Relationship between Oxidation Reduction Potential (ORP) and Volatile Fatty Acid (VFA) Production in the Acid-Phase Anaerobic Digestion Process

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Contents

Abstract

The purpose of this research was to investigate the relationship between the oxidation-reduction potential (ORP) measurement and volatile fatty acid (VFA) production in the acid-phase anaerobic digestion process under different conditions of temperature and residence time. Two identical anaerobic digesters were operated while VFAs, SCOD, VSS, alkalinity, ORP and pH were measured. In digester 1, VFA production of 5,556 mg/L was generated with an ORP of -315 mv at a 10 day SRT; while 5,400 mg/L of VFA with an ORP of -389 mv was recorded in digester 2. The SRT was adjusted at 5, 8, 10, 12 and 15 days and the optimum SRT was 10 days in both digesters. The results of this study indicate there were no tight relationship between VFA production and ORP values, thus ORP by itself is not a good predictor of the amount of VFAs generated. However, ORP combined with temperature had good linear relationship with VFA production. An ORP range of -315 to -390 mv was desirable for maximizing VFA production in both anaerobic digesters. Different temperatures (14, 29 and 37 ℃) were trialed and the results indicate that the conditions at 29 ℃ and 37 ℃ were not significantly different in terms of VFA production, however, less VFAs were generated at the lowest temperature of 14 ℃.

Dedication

This thesis is dedicated to my wife Seoung Ok Lee and my son Jong Yoon Lee who have given me a lot of love at all times. Seoung Ok Lee, you have been with me every step of the way, through good times and bad. Thank you for all the support and your decision to postpone your studies in order to look after me and our lovely son Jong Yoon. Thank you very much and I love you.

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1 Introduction

1.1 Acid-phase anaerobic digestion and volatile fatty acids

The anaerobic process has been used for the treatment of many complex organic wastes including municipal, chemical and agricultural wastewaters (George and Peter 1998; Gavala et al. 1999; Largus et al. 2004; Gomez et al. 2005; Nguyen et al. 2006; Ramakrishnana and Gupta 2006). The anaerobic process has advantages over aerobic treatment systems including the production of less sludge, generation of useful biogas, overall pathogen removal, lower energy consumption and lower space requirements (Sharma and Pellizzi 1991; Malina and Pohland 1992; De Sousa and Foresti 1996; Demirer and Isci 2006).

The anaerobic treatment process can be divided into two metabolic phases, an acid-phase and a methane forming-phase. After Pohland and Ghosh (1971) postulated the concept of phase separation, many studies have been conducted to find the optimum environmental conditions for each bacterial group in order to enhance the overall process stability and the control of each phase (Chyi and Dague 1994; VanLier et al. 1996; Elefsiniotis et al. 2005). In particular, acid-phase anaerobic digestion has increased in importance as compared to the methane forming-phase since volatile fatty acids (VFAs) are generated as products from acidogenesis and these can be used in biological nutrient removal systems (Akın and Ugurlu 2005; Elefsiniotis and Wareham 2007).

The bacteria responsible for biological nutrient removal in a wastewater treatment process require soluble organic products for energy to maintain life and produce new biomass. However, some wastes (e.g. wastes from the paper industry) often do not contain sufficient easily biodegradable organic matter to meet effective biological nutrient removal requirements. In this case, additional soluble carbon sources generated internally (in the anaerobic zone) or added externally can solve this problem. An external carbon source such as VFAs, but also methanol, ethanol, or other wastewaters with a high soluble COD concentration (e.g. a wastewater from a food processing factory) could be used. In a nutrient removal system the use of VFAs as external carbon

sources produced via acid-phase anaerobic digestion has increased in importance (Brinch et al. 1994). This is because naturally generated VFAs have significant benefits over other sources due to economics and the ease with which they are generated (Elefsiniotis et al. 2004).

The amount and speciation of VFAs produced by acid phase anaerobic digestion can vary as a function of environmental and physical parameters such as HRT, SRT, pH and temperature, etc. For example, the HRT affects the amount and type of substrate being used by the microorganisms. Previous research has been performed on the optimization of the acid-phase step, and an optimal HRT of 12 hours was reported based on the degree of VFA production, TOC solubilization and organic substrate degradation (VSS reduction) in a continuous-flow UASB reactor (Elefsiniotis et al. 1996). In contrast, the SRT affects the generation time and type of organisms predominating in a digester. Several studies have shown the effect of SRT on the acid phase of primary sludge digestion. For example, Elefsiniotis et al. (1996) indicated that biodegradation in terms of specific TOC solubilization rate and VSS reduction seemed independent of SRT from 10 to 20 days However, the net production of VFAs was clearly dependent on SRT. More VFAs were generated with longer SRTs, that is, slower growing organisms which can better metabolize substrate can be developed to generate VFAs at the longer SRTs (Elefsiniotis et al. 1996).

In another study on a starch-rich industrial wastewater, an optimal ratio (1:1) of industrial to municipal wastewater was found to have the maximum VFAs and the most SCOD production (Elefsiniotis et al. 2005). VFA production was almost double compared to municipal wastewater because starch-rich wastewater has a significant amount of carbohydrates that are normally easier to biodegrade than lipids and proteins. The result was a high degree of hydrolysis, acidogenesis and more VFAs in terms of net SCOD, specific SCOD production rate, VFA:SCOD ratio and VSS reduction. It was therefore clear that VFA production was greatly enhanced through the addition of a starch wastewater into an anaerobic municipal wastewater digester.

The effect of HRT and low temperature on VFA production has also been investigated, and a 30 hour HRT and 25 ℃ was indicated as the point of

highest concentration of VFAs and soluble COD and specific production rate (Maharaj and Elefsiniotis 2001). Their study indicated that VFA production was feasible at low temperatures (8 ℃), particularly in the case when industrial wastewater was added. Temperature variation can change bacterial population levels in an acid-phase anaerobic digester and low temperatures can make VFA production rates decrease. However, stable operation and high levels of VFAs (280 mg/L net VFAs as acetic acid) were observed in the previous study. Banerjee et al. (1998) also investigated temperature ranges with an HRT of 30 hours. In their study 25 ℃ had the highest net VFA concentration in the temperature range investigated (20 - 35 ℃).

Early researchers (Zoetemeyer et al. 1982) investigated temperature effects on acid-phase anaerobic digesters. They found that in the mesophilic range, butyrate dominates at all temperatures. Ethanol is an important product at low temperatures and lactate becomes a significant product beyond the optimal mesophilic temperature, however, butyrate was the main product in the whole mesophilic range at high sludge loadings (Zoetemeyer et al. 1982).

The influence of pH (from 4.5 to 7.9) was studied by Zoetemeyer et al. (1982) with respect to maximizing VFA concentration. A pH range of 5.7 - 6.0 was recommended as the optimum pH to produce VFAs. According to their study, the products of the acid-phase anaerobic digester were butyric, propionic, acetic, formic, lactic, and ethanol. The amount of these products was dependent on the dilution rate and, more strongly, on the culture pH value. They also indicated that selecting an appropriate pH value can target specific major VFA products. For example, with a pH value set to 5.7, butyric acid was the main product formed at all dilution rates, while propionic acid decreased. At another pH value of 6.4, propionic acid was not detected at dilution rates greater than 0.1, while the butyric acid concentration decreased and the concentration of lactic and formic acid increased. The acetic acid concentration was however stable over a large range of dilution rates.

Hydrogen gas production can be increased by maximizing VFAs generated in the acid phase anaerobic digester, since anaerobic digestion of organic matter yields VFAs as fermentation end products along with hydrogen. For example, the butyrate-acetate fermentative route has been widely

accepted for anaerobic hydrogen production in a mixed culture system (Cheoug et al. 2007). The stoichiometric reactions are shown below (Cheoug et al. 2007):

$C_6H_{12}O_6 + 2H_2O \rightarrow \mathbf{2CH_3COOH} + 2CO_2 + \mathbf{4H_2}$

$C_6H_{12}O_6 \rightarrow \mathbf{CH_3CH_2CH2COOH} + 2CO_2 + \mathbf{2H_2}$

$C_6H_{12}O_6 + H_2O \rightarrow C_2H_5OH + \mathbf{CH_3COOH} + 2CO_2 + \mathbf{2H_2}$

$4C_6H_{12}O_6 \rightarrow \mathbf{2CH_3COOH} + \mathbf{3CH_3CH_2CH_2COOH} + 8CO_2 + \mathbf{8H_2}$

Since hydrogen gas has energy and is non-polluting, the H_2 yield and production rate are also of interest in addition to the VFA concentration. Fan et al. (2005) found that the concentration of total VFAs increased with increasing HRT from 18 to 48 hr and reached 2.7 g/L at an HRT equal to 48 hr. In addition, a pH of 5.5 was the optimum value for maximum H_2 production, while the VFA production significantly decreased at pH values of 6.0 and 6.5. Cheong et al. (2007) studied the conversion of a carbohydrate-rich synthetic wastewater with variations in pH and HRT in an anaerobic sequencing batch reactor. A high hydrogen production rate of 4,460 - 5,540 mL/L/day was obtained at an HRT of 8 hr and a pH of 5.7 (Cheoug et al. 2007). An HRT of 8 hr was the optimal region for the hydrogen production rate; however it was found that there was no significant effect on the hydrogen production rate with variations in pH.

1.2 Monitoring and control methods for VFA production other than ORP

To control biological treatment systems, many control parameters (e.g. pH, temperature, SRT, HRT, alkalinity and dissolved oxygen (DO)) have been considered and each has their own distinct advantages and disadvantages. For example, DO control is used for many aerobic microbial processes; however, one problem with DO control is that DO readings become unreliable when the DO is below 0.1 to 0.2 mg/L (Moriyama et al. 1993). Thus, under low oxygen tensions, using DO for control and monitoring is not very useful.

Alkalinity has also been used for determination of total VFAs in acid-phase anaerobic digesters (Anderson and Yang 1992); however, alkalinity was not easy to set up as on-line system for control. On-line titration of VFAs was developed for the process control of an anaerobic digester (Fuerhacker et al. 2001); however, this method was much more complicated than other methods.

Wilcox et al. (1995) developed a neural network system based on bicarbonate monitoring since both bicarbonate alkalinity and pH are good environmental indicators of the state of the acid-phase anaerobic digestion process. VFAs, the main products in the system, will drop the pH and lower the buffer capacity. In order to detect faults in the process, the authors combined several on-line sensors (pH, H_2, CO_2, gas flow and bicarbonate alkalinity), with data from the sensors collected every two minutes and evaluated by a neural network simulation program. The results suggested that the process was very sensitive to bicarbonate alkalinity changes and that the neural network system was capable of rapid recognition of sudden changes in VFAs concentrations. The system could also be operated in real time to control the VFA production through the use of a bicarbonate dosing pump.

On-line capillary gas chromatography has also been successfully developed and applied in industrial applications to monitor VFAs in anaerobic digesters (Diamantis et al. 2006). However, gas chromatography systems are not easy to maintain due to their complexity and expense (e.g. the column is costly to replace).

Another monitoring method for VFAs is a biosensor based on nitrate reduction (Rozzi et al. 1997). In this system, nitrates are determined by measuring the acid equivalents added by a titrator to neutralize the hydroxyl ions produced by the denitrification reaction in the presence of an organic carbon source in excess. VFA concentrations are determined as readily biodegradable COD (rbCOD) and a satisfactory correlation ($R^2 > 0.9$) between organic carbon and reduced nitrate has been found. VFAs are indirectly measured by rbCOD since the prevailing fraction of rbCOD in digested anaerobic liquors is primarily made up of VFAs. The authors indicated that this method can be easily automated and might allow a VFA determination every 20 – 40 minutes, which is sufficient for many anaerobic applications.

Feitkenhauer et al. (2002) also used a reliable on-line titration method to measure the concentration of VFAs without using expensive analytical devices such as a GC. A titration cell was designed and built to suit the specific needs of the VFA titration without biomass separation. Titration results and GC determination of VFAs showed good agreement between the two methods (total amount of VFAs were 31.75 mmol/L by GC analysis and 34 mmol/L by using their on-line titration method). Reproducibility and long–term stability of the VFA measurements were sufficient to ensure several days of operation without maintenance. The authors indicated that this method could be adapted to full scale anaerobic digestion plants.

A fundamental relationship between pH and VFA concentration was investigated by Munch and Greenfield (1998). The authors proposed that measured pH values could be used to estimate the corresponding VFA concentrations in an acid-phase anaerobic digester. The estimation of VFAs from a single pH measurement would be useful since pH is a parameter that is relatively easy to measure as compared to a biosensor or gas chromatograph. A mathematical model was developed to predict VFA concentrations and an increase of around 30 mg/L of VFA for each 0.1 drop in pH was reported. However, there were several assumptions behind the model, the main one being that there was no significant metabolism-generated alkalinity apart from that caused by VFA production. Depending on the wastewater source, there could be circumstances where additional metabolism such as protein breakdown could be a potential source of alkalinity. However, if the total alkalinity for a particular system remains constant and only the relative contributions of bicarbonate and VFAs are changing, this model could be acceptable under any conditions of wastewater.

1.3 ORP and anaerobic digestion

ORP can be a useful parameter to control anaerobic digesters since it measures the net value of all complex oxidation-reduction reactions within an aqueous environment. Many complex reactions occur in an anaerobic sludge digester and these are not always easy to identify separately. Some biological reactions occur together with bacterial chemical reactions while some

products from previous biological reactions can be used as substrates for subsequent reactions. Although, there are many anaerobic biological reactions occurring (with many of them difficult to understand) the measurement of ORP has been used in several instances as an environmental parameter in anaerobic digestion systems (Blanc and Molof 1973; Ishizaki et al. 1974; Colmenarejo et al. 2004; Akın and Ugurlu 2005).

In particular, Blanc and Molof (1973) indicated that an increasing ORP correlated to inhibited or decreasing levels of digester performance. They observed that under normal anaerobic digestion conditions, the measured ORP value in the sludge remained constant in an operating range of -220 to -290 mv (E_H). This optimum ORP range was recommended to maintain stability of anaerobic digesters in their study. Peddie et al. (1990) investigated the ORP transition under aerobic, anoxic and anaerobic conditions controlled by reaeration and deaeration in an aerobic sludge digester. The different ORP profiles for aerobic, anoxic and anaerobic tensions were observed and correlated to the slope changes in the ORP. The reproducibility of the ORP profile and sensitivity of the measured potential made ORP an ideal parameter for auto monitoring and process control. Although Peddie et al. (1990) studied aerobic sludge digestion, they indicated that ORP monitoring under anaerobic conditions could be used extensively because of its utility over the full range of redox conditions.

Two different types of pilot-scale fermentation reactors, a fixed-bed reactor and a suspended biomass reactor were investigated for total volatile fatty acids production (Colmenarejo et al. 2004). The authors indicated that the ORP values were not significantly influenced by the type of reactor and although the ORP was the same (around -300 mv) in both reactors, the total volatile fatty acids formation was slightly higher in the support zone of the fixed-bed reactor than in any other zone in that reactor as well as in the suspended biomass reactor. This phenomenon could be attributed to the utilization of the support media in the fixed-bed reactor which contributed to an increase in the rate of organic matter assimilation and degradation. Within the suspended biomass reactor (without support) the degradation was limited by the contact between the microorganisms and the substrate. The relationship between ORP and HRT was also explored in both reactors and

the following equation was developed.

$$\mathbf{ORP = - 267.6\ [ln\ (HRT)] - 32.1\ (R^2 = 98\%)}$$

This equation indicates that the ORP decreases as the HRT increases in the range (HRT: 0 ~ 6 hr).

Recent research on acid-phase anaerobic digestion has shown the possibility of using ORP to control the production of a certain species of VFAs For example, Wang et al (2006) used 10 mM $FeCl_3$ as an oxidation reagent to adjust the ORP in an anaerobic reactor. The reactor without ORP control dropped gradually from an initial value of 100 mv down to a final value of -350 mv; however, after adding $FeCl_3$ the ORP increased from -350 to -280 mv. Propionic acid was generated following the ORP increase. The authors indicated that an ORP of more than -150 mv always led to propionic acid-type fermentation at any pH and, at a pH of about 5.0, either propionic acid or butyric acid fermentation might occur depending on whether the ORP value was high or low. More propionic acid was generated at the higher ORP values. In a separated two-phase anaerobic treatment system, the accumulation of propionic acid in the acid-phase anaerobic digester is not the best environment for methane production because the acetogenic rate of ethanol and butyrate by hydrogen-producing acetogensis is relatively higher than that of propionic acid (Gallert and Winter 2006; Wang et al. 2006). In summary, an ORP condition of more than -150 mv should not be operated in an anaerobic digester to avoid propionic acid-type fermentation (Wang et al. 2006).

ORP as a controlling and/or monitoring parameter has been tried for many other different treatment systems such as high-sulfate wastewater treatment systems (Khanal and Huang 2003; Khanal and Huang 2006), activated sludge systems (Li and Bishop 2004) and nutrient removal systems (Kishida et al. 2003; Akın and Ugurlu 2005). In particular, Khanal and Huang (2003) investigated the usefulness of ORP to control sulfide, since high sulfide concentrations can often lead to poor performance and eventual process failure of anaerobic treatment system. They found that when oxygen was added to raise the ORP from -230 to -180 mv, the dissolved sulfides were reduced to undetectable levels at all influent sulfate concentrations (Khanal

and Huang 2003). In addition, the entire performance of the anaerobic treatment system was improved in terms of methane yield because of the activity of facultative bacteria.

ORP has also been used to control and monitor SCOD production during hydrolysis through the addition of chemical agents. Chang et al (2002) studied the relationship between the change in ORP value and increments in SCOD during hydrolysis with waste activated sludge by dosing different concentration of NaOH. The authors developed a relationship between SCOD and ΔORP (i.e. the difference of ORP values at 5 min (ORP_{5min}) and at time t (ORP_t)). They suggested that the increase in SCOD could be predicted from ORP values even though the concentrations of total suspended solids in the reactors were different. A liner relationship was developed as shown below:

$$\mathbf{SCOD = 11.9 \times \Delta ORP + 1762.7 \ (R^2 = 96\%)}$$

The NaOH added in their study was used to enhance the rate of solubilization of wasted sludge. However, chemical pretreatment methods are not common methods of increasing the biodegradability in biological anaerobic digester systems, since strong alkaline chemical agents can destroy the cell wall of anaerobic bacteria. In particular, high concentrations of amino acids were observed in their study after alkaline pretreatment and this reflected cell wall destruction. Moreover, it is generally accepted that a high sludge pH would have to be neutralized before sludge disposal.

ORP has been used successfully as a control and monitoring parameter in many anaerobic treatment systems (Colmenarejo et al. 2004; Wang et al. 2006) primarily because organic material under anaerobic conditions is subjected to degradation by redox reaction catalyzed enzymes. The total VFAs may therefore have a relationship with the ORP value since VFAs are major products from biological reactions in acid phase anaerobic digestion.

To use the ORP as a monitoring and control parameter, the response time of platinum electrodes is critical and it is sometimes slow (Gupta et al. 1994; Minton and Molof 1999). Minton and Molof (1999) developed a rapid, reliable method of establishing the electrode potential by testing with chemical pretreatment methods. Test electrodes were exposed to various chemical

agents and were either inserted immediately into the digesters or allowed to dry in air before insertion. The authors indicated that chemical pretreatment by stannous chloride and thioacetamide/hydrogen sulfide (followed by a drying procedure in air) showed the best response (rapid and stable ORP). The ORP reached 85 to 87% of the 24-hour reading in 20 minutes. Another study using a flow-through cell for ORP measurement in anaerobic systems yielded a stable response within 30 min of commencing the measurement (Gupta et al. 1994). This indicated that most of the random errors (e.g. biofilm growth on the probe and measurement fault due to exposure to oxygen during the cleaning procedure) were eliminated by this method.

1.4 Research objectives

This research ……

- Examines the relationship between the ORP range and the amount of VFAs produced in order to see if ORP is a suitable monitoring parameter for VFA production. ORP may change as a function of VFA production or at least the value of ORP may indicate the amount of VFAs.

- The optimum value of ORP for maximum VFA production needs to be investigated, since an ORP range may exist correlated to maximum VFA production.

- VFAs are generated as a function of varying physical and environmental parameters. HRT/SRT and temperature are common control parameters in anaerobic digestion systems and operating under different conditions may influence the hydrolysis of organic matter and its conversion to VFAs. Therefore, this research will also investigate the relationship between the ORP range and VFAs under conditions of varying parameters (i.e. HRT/SRT and temperature, etc). The values of HRT/SRT will be 5, 8, 10, 12, and 15 days since these are typical values of HRT/SRT for anaerobic digestion. The temperature will be controlled at 14, 29 and 37 ℃ (with a 10 day HRT/SRT) since of interest is the operation covering a wide spectrum of temperature.

2 Materials and Methods

2.1 Materials

2.1.1 Soy flour as a feed source

Soy flour was used as the feed source in this experiment and it was full-fat soy flour manufactured by the company Cereform Ltd. located in Australia. The soy solution for the feed was made up the day before allowing some VFAs to be generated before the feeding process was conducted. The characteristics of the feed soy solution are shown in Table 2.1 below.

Table 2.1 Characteristics of the feed soy solution

Parameters	Mean	Standard Deviation
pH	4.2	1
ORP (mv)	23	5
VFAs (mg/L)	800	200
Alkalinity (mg/L as $CaCO_3$)	0	0
VSS (mg/L)	26,879	2,187
SCOD (mg/L)	8,676	596
Temperature (℃)	20	3

The U.S Department of Agriculture has reported that the composition of full-fat soy flour is 35 % protein, 35 % carbohydrate and 21 % fat (refer to appendix A.1). In contrast, organic matter found in domestic wastewater is about 40 % protein, 25 - 50 % carbohydrate and 10 % fats and oils (Metcalf and Eddy 2003). Defatted soy flour would have less than 21 % fat which would be similar to that in a domestic wastewater; however, in this study, full-fat soy flour was chosen because it was inexpensive and easy to treat and store. Moreover, its biodegradability was excellent.

2.1.2 Seed

The digester was seeded with 10 L of digested sludge obtained from the Christchurch Wastewater Treatment Plant located at Bromley, Christchurch,

New Zealand. The initial 10 L of digested sludge was added to 10 L of 40 g/L of soy flour solution and after that the digester was sealed. The flow through the pump was closely monitored for the first 4 to 6 hours and valve blockages were removed manually by removing the valve, sealing the pipe with a rubber bung, clearing the blockage, removing the bung and reconnecting the valve. The pump was then switched on continuously to circulate the biomass. Another identical anaerobic reactor was set up with the seed source comprising 4 L of the waste solution from the previous reactor and 16 L of 40 g/L of the soy flour solution.

2.1.3 Anaerobic reactors

Two identical stainless steel laboratory-scale anaerobic reactors were set up, each having a volume of 30 L. The stainless steel cylinder had a sealed base and a removal lid, while a grease and rubber seal generated an airtight seal to operate under anaerobic conditions. An external pump was used for continuous mixing, removing contents from the bottom of the reactor and returning it to the top of the reactor. The pump was running continuously to allow mixing completely except during the feed and waste process. When the feed and waste process was conducted, the switch off the pump or the valve of the inlet/outlet pump was turned off. After finishing the feed and waste process, the switch or valve was turned on such that the whole feed/waste process took about 5 minutes. Gas produced in the system was vented out from the reactor headspace and released through a water trap so that no oxygen could enter through the ventilation tube.

An ORP probe was inserted into the reactor through a hole in the top of the reactor. The hole was blocked by a rubber stopper so that no air could enter, however when the ORP probes were taken from the reactor to be cleaned, another identical rubber stopper was temporarily used to block the hole to keep anaerobic conditions. ORP was monitored by a Labview program allowing real time monitoring. ORP data saved automatically to the computer hard drive ready to be evaluated via Excel. A schematic of the two anaerobic reactors is shown in Figure 2.1.

Both reactors were maintained at a 10 day SRT for a month and then connected together for another month so that identical conditions prevailed

before the first run was conducted. Whenever two reactors were connected together, a 10 day SRT was maintained.

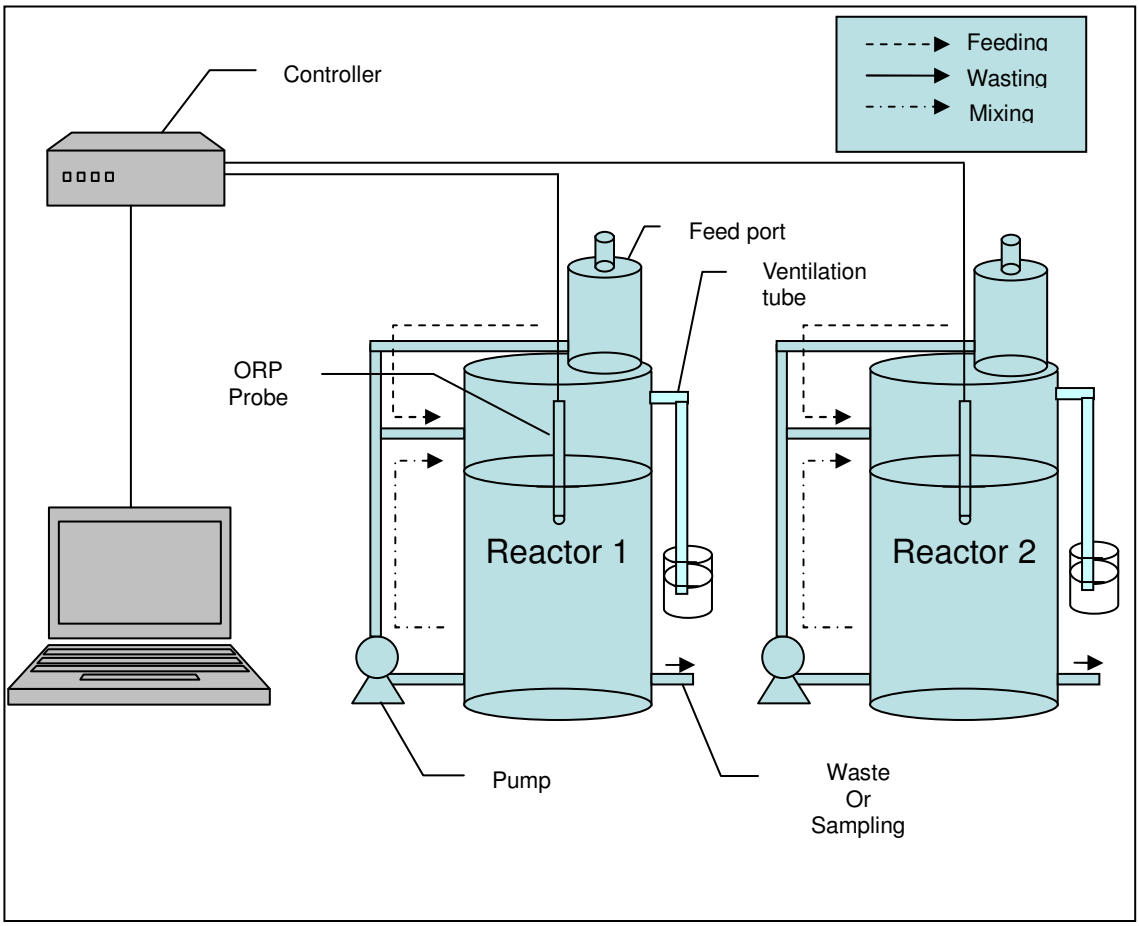


Figure 2.1 Schematic of two anaerobic reactors

2.2 Methods

2.2.1 Operating conditions

This experiment was divided into 2 stages depending upon the control strategy. Stage 1 was HRT/SRT control while stage 2 was temperature control. Each run was conducted for 2.5 SRTs to ensure steady-state conditions. Between each run, the two reactors were connected together for 7 days (also at a 10 day HRT/SRT) to ensure identical conditions for both reactors. The operating conditions of both reactors are shown in Table 2.2.

Table 2.2 Operating conditions at steady state

Stage / Run		HRT/SRT control (days)	Temperature control (℃)
Stage 1	1	10	29
	2	5	29
	3	15	29
	4	12	29
	5	8	29
	6	10	29
Stage 2	7	10	37
	8	10	14

2.2.2 Feed and waste process

A certain amount of soy solution was fed everyday to maintain a constant HRT/SRT (i.e. 80 g soy flour was added into 2 L water). With respect to feeding, the pump for the circulation of contents was stopped or valves at the inlet/outlet of the pump were closed. The feed solution was then poured into the port located in the top of the reactor, and after that, the valves for feeding and wasting were opened slowly at the same time. The feeding and wasting procedure was finished at the same time to prevent unbalanced pressure being created between the inside and the outside of the reactor. After feeding and wasting was finished, 100 mL of sample was taken from the waste solution to measure several environmental parameters. Because the anaerobic system had no recycle, HRT and SRT were identical.

2.2.3 Sample preparation

Samples for all parameters (VFAs, SCOD, VSS and alkalinity) were directly collected from the waste solution. Approximately, 100 ml of sample was collected from the waste solution and 50 ml of that was used to determine alkalinity. The rest of the sample was filtered with a glass micro fiber filter (GF/C) and a 0.45 μm filter paper to prepare the sample for VFA analysis by gas chromatograph, SCOD and VSS. Standard methods (APHA-AWWA-WEF 1989) were employed to determine all parameters, except for VFAs.

2.2.4 Gas chromatography

The concentrations of volatile fatty acids (VFAs) in the sample were measured by gas chromatography. Acetic (HAc), Propionic (HPr), n-Butyric (n-HBu) and n-Valeric (n-HVa) acids were the major VFAs found in this study and these were identified by comparing the retention time with that of known standards. The amount of each acid present was quantified by comparing the areas under the peak of the chromatogram to that of known standards. The conditions of the gas chromatograph are listed in Table 2.3.

Table 2.3 Gas chromatographic condition for the determination of VFAs

Column	Capillary Column (HP 19091N-133) HP-INNOWax Polythylene Glycol
Column Temperature	120-250 ℃ min^{-1} with 10 ℃ min^{-1}, after that 250 ℃
Injector Temperature	280 ℃
Injector volume	1μm
Carrier gas	He
Split	1:40
Detector	FID
Analysis time	18 min
Retention time	Acetic acid 2.92 min
	Propionic acid 3.54 min
	n-Butyric acid 4.27 min
	n-Valeric acid 5.26 min

2.2.5 ORP

The ORP probe used in this research was manufactured by the company YSI and the number of the model was “115 – 1”, as the accessory part of the ‘pH 100’ model. The range of the ORP measurement was reported to be -1999 mv to +1250 mv with an accuracy of ± 0.1 % ± 1 digit. The reference electrodes were Ag/AgCl and 3.5 M KCL gels, while the redox electrode was platinum.

The most common problem for ORP determination is that readings can

be different even though the probes are in the same solution. In addition, after the probes are used for a certain time in anaerobic conditions, one probe can take a longer time than another to yield a stable ORP value. To assist in minimizing this problem, the probes were checked in a quinhydrone buffer solution frequently following the method specified by the manufacturer of the probes and as suggested by other researchers (Khanal and Huang 2003). This technique is not a calibration per se and all that can be said is that the electrode will read a correct value in a saturated quinhydrone solution under aerobic conditions (Molof 1996). There remains no standard procedure for measuring ORP and calibrating under in anaerobic conditions.

The cleaning of the platinum sensing surface was conducted frequently with a water bottle and a soft cloth in order to prevent reading errors caused by sludge coating. However, it has been indicated by the manufacturer that 8 to 24 hours are required to provide stable readings after the platinum sensing surface has been cleaned. In this research, data produced from trial experiments indicated that 10 to 12 hours was required to reach a stable ORP value. The cleaning of the platinum sensing surface was conducted just after finishing the feed/waste procedure in order to minimize the effect on the ORP values from being cleaned by pure water (which has a high positive ORP value compared to the negative ORP values under anaerobic conditions). A stable representative ORP reading was taken just before the feeding and wasting procedure and also just before cleaning the probes.

2.2.5 Operating problems

Maintaining the volume of the digester

To maintain a certain SRT/HRT in each run, the same amount of solution was fed once a day continuously through each run. Theoretically, because the same amount of soy solution was used for both feeding and wasting, a constant 20 L of contents was expected to be maintained in the digester. However, during the experiments, it became apparent that some of the volume of the digester contents was lost. This could be because gas (mainly hydrogen, carbon dioxide and hydrogen sulphide) was released through the ventilation tube (gas bubbles releasing were observed on occasion). Although, hydrolysis and acidogenesis are initial steps in the anaerobic process, gases

could be released by anaerobic bacterial reactions during these steps as shown in Figure 2.2.

Fermentation

$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + \mathbf{2CO_2}$

$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + \mathbf{2CO_2} + \mathbf{4H_2}$

$C_6H_{12}O_6 + \mathbf{2H_2} \rightarrow 2CH_3CH_2COOH + 2H_2O$

$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + \mathbf{CO_2} + \mathbf{3H_2}$

$C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$

Sulphate Respiration

$2CH_3CHOHCOOH + SO_4^{2-} + H^+ \rightarrow 2CH_3COOH + 2CO_2 + 2H_2O + \mathbf{HS^-}$

$2CH_3CHOHCOOH + 3SO_4^{2-} + 3H^+ \rightarrow 6CO_2 + 6H_2O + \mathbf{3HS^-}$

Figure 2.2 Example reactions of the anaerobic process

Another possibility could be evaporation that could happen through the ventilation tube and the level indication tube. Although the small surface area in contact with the ambient air would seem to mitigate this, evaporation could be accelerated because of the high temperature of the contents compared to the temperature outside of the digester.

To minimize volume loss, a procedure to set the volume at 20 L was implemented between each run. That is, the volume was checked and if the volume was less than 20 L, a certain amount of the feed solution would be added to fill the reactor up to 20 L. To check the exact volume of the reactor, the two pumps for circulation were stopped temporarily to make the level stable. The valves for ventilation were then opened to allow the pressure inside and outside of the reactor to equilibrate. After that, the level of the reactors was taken to identify the exact volume. This procedure allowed a decision to be made as to whether the amount of feed solution needed to be increased.

The use of two digesters

Operating two digesters meant that two runs under theoretically different conditions could be conducted simultaneously in order to save experimental time. Thus, at the beginning of this research, a base run (at a 10 day SRT) was conducted; however, after the two digesters were separated, the results of all chemical parameters indicated small differences between digester 1 and digester 2. This means that the two digesters were not identical, even though they were at the same SRT. This may have been due to initial slight differences between the two digesters (e.g. the circulation rate, variations in the ORP probes themselves, a slightly different digester size, the amount of feed and waste solution and different temperatures at different spots in the lab).

Although the initial idea was to investigate the two digesters under different conditions, in fact they were operated under similar conditions. This allowed trends in parameters to be investigated (such as VFAs, ORP, SCOD, VSS and pH) between the two digesters. Whenever an individual run finished, the two digesters were connected together for a week to allow mixing of the contents completely before the next run was started.

The increase in alkalinity

The alkalinity of an anaerobic system is important because it represents the buffering capacity of the system (i.e. the capacity to resist variations in pH). In the acid-phase anaerobic digestion, alkalinity is expected to decrease because VFAs and carbon dioxide are generated.

In this experiment, the initial pH of the feed solution was approximately 4.2, while the pH of the waste solution was around 4.8. The low pH of the feed solution can be explained by the fact that some VFAs (800 mg/L as acetic acid) were generated overnight and these initial VFAs would consume alkalinity in the feed solution. Alkalinity depends on the end-point pH used and a pH of 4.5 was chosen for the end-point pH in this experiment (APHA-AWWA-WEF 1989). Thus the alkalinity of the waste solution was able to be measured because a pH of 4.8 was higher than 4.5 while no alkalinity was measured in the feed solution due to the pH of 4.2. This increase in alkalinity in the digesters possibly can be explained by the simultaneous presence of ammonia and bicarbonate. That is the formation of NH_4HCO_3 through the

following equilibrium:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$
$$HCO_3^- + NH_4^+ \leftrightarrow NH_4HCO_3$$

The presence of this salt can put alkalinity in the digester, even though high concentrations of VFAs are present (Alvarez 2003).

The effect of heat generated by circulation pumps

A digester was connected with a pump to mix the contents completely. The pump was an electric pump connected with PVC pipe to the digester. This circulation pump generated substantial heat causing the temperature of contents in the digesters to be raised. For example, the digesters operating at ambient temperature (approximately 20 ℃) was monitored and the contents measured to be approximately 29 ℃ during the experiments. To ensure the pumps were not over heating before the run at 35 ℃ was conducted, the pump had to be in flowing cool tap water. The actual temperature of contents in the temperature control room at a run with 35 ℃ was approximately 37 ℃. Similarly, the actual target of low temperature was 8 ℃; however, 14 ℃ was the lowest temperature that could be maintained for the digester contents. This was even though an ambient temperature was maintained at approximately 7 ℃ and the pumps were located outside of the temperature control box to minimise the effect of heat generated by pumps.

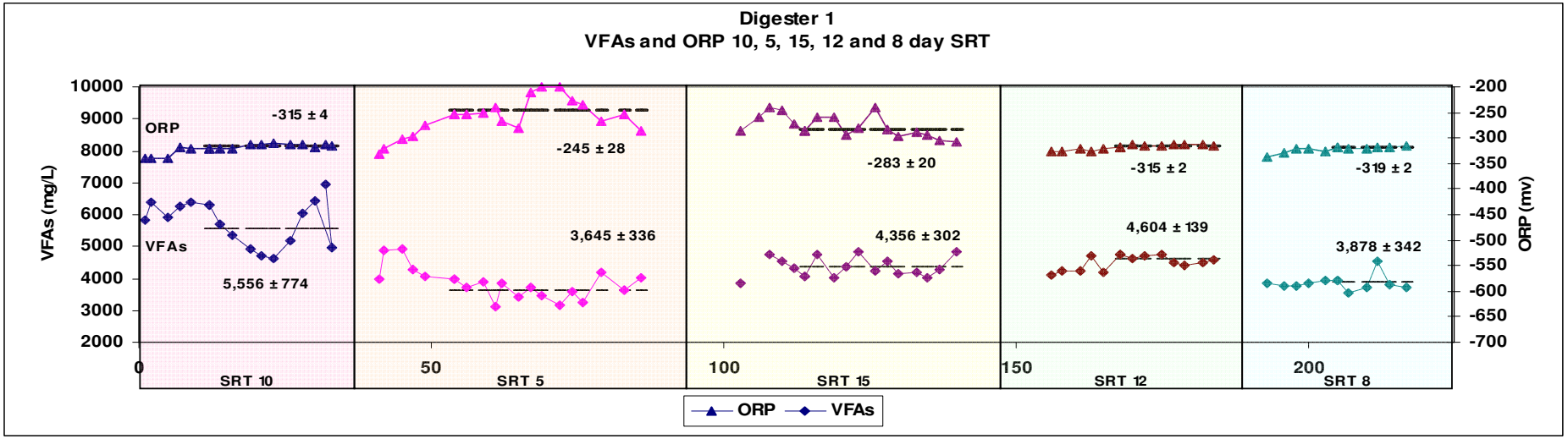
3 Results and Discussion

3.1 Relationship between VFAs and ORP as a function of SRT

The raw data for VFAs and ORP for the entire experimental period are shown in Figure 3.1, while the mean VFA and ORP values for digester 1 as a function of SRT (5, 8, 10, 12 and 15 days) are shown in Figure 3.2. A run period of more than 2.5 SRTs was selected to stabilize the parameter values since steady state has been normally achieved in less than 2 SRTs in other process studies (Elefsiniotis et al. 1996). Since fluctuations in the data were observed between each SRT condition, the initial 5 data points for each SRT were ignored in order to eliminate carry-over effects from the previous SRT run. These initial data points were discounted for all parameters investigated.

VFAs measured in this experiment include acetic, propionic, butyric and valeric acid. The concentrations of propionic acid and butyric acid were converted into equivalent concentrations of acetic acid in proportion to their molecular weights, thus the total VFAs were expressed as acetic acid. Valeric acid was not added into the VFAs because of its low production as compared to the others.

In terms of anaerobic degradability in an acid phase anaerobic digester, conditions of high concentration of VFA are the most desirable. The maximum VFA of 5,556 mg/L was generated at a 10 day SRT making this the optimum SRT for the conditions of this research. In contrast, a minimum VFA value (3,645 mg/L) in digester 1 was generated at a 5 day SRT. An ANOVA test analysis was conducted to identify whether there were significant differences in the mean VFA production between each SRT condition and the results are shown in Table 3.1. The F value had to exceed an F critical value in order for there to be a significant difference between VFA values of each run at a probability (p-value) of 0.05. According to Table 3.1, the F value of 30.5 exceeded the F critical value of 2.6 with a probability of 2.7×10^{-12}. Therefore, there were highly significant differences between VFA production under the conditions of 5, 8, 10, 12 and 15 day SRT.



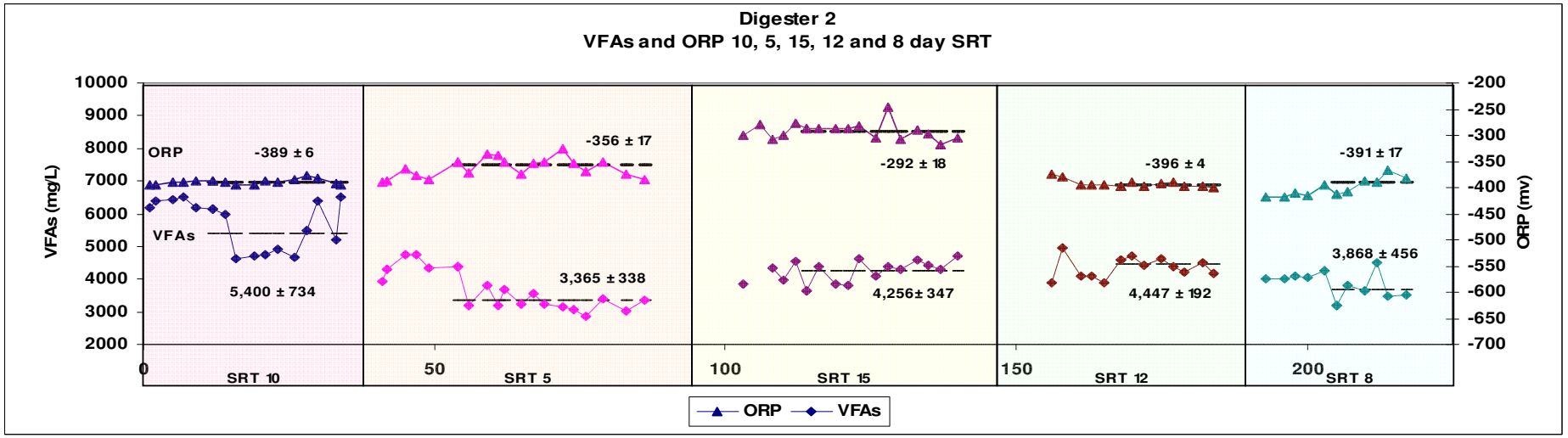


Figure 3.1 VFAs and ORP in digester 1 and 2 as a function of SRT for the entire experimental period

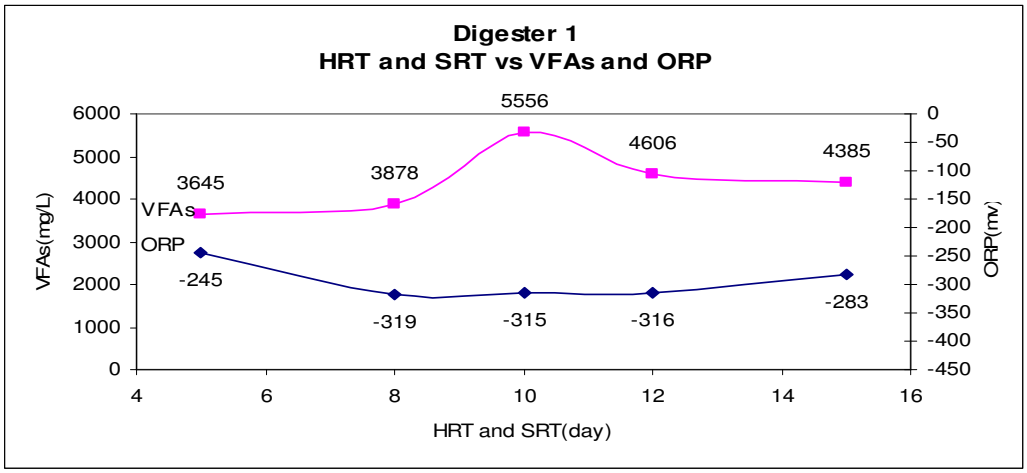


Figure 3.2 VFA and ORP under different SRT conditions in digester 1

Table 3.1 ANOVA test with VFA values for digester 1

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	24658687	4	6164672	30.5	2.7E-12	2.6
Within Groups	9097153	45	202159			
Total	33755840	49				

Hydrolysis is characterized by ORP values of around -300 mv (Colmenarejo et al. 2004) and as seen, the minimum ORP value was -319 mv at an 8 day SRT while the maximum ORP was -245 mv at a 5 day SRT. ORP values at a 10, 12 and 15 day SRT were -315 mv, -316 mv and -283 mv respectively. The ORP values could reveal that particulate material is being more hydrolyzed to simple monomers at an 8, 10, 12 and 15 day SRT than at a 5 day SRT.

The results of an ANOVA test for the ORP values at an 8, 10 and 12 day SRT are shown in Table 3.2. The results indicate that the F value of 3.3 was lower than the F critical value (3.4) with a probability (0.056) which is higher than 0.05. This means that there was no significant difference between ORP values at the 8, 10 and 12 day SRTs.

Table 3.2 ANOVA test of ORP values at an 8, 10 and 12 SRT for digester 1

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	65.8	2	32.9	3.3	0.056	3.4
Within Groups	219.5	22	10			
Total	285.4	24				

The VFAs and ORP values of digester 2 as a function of SRT are shown in Figure 3.3.

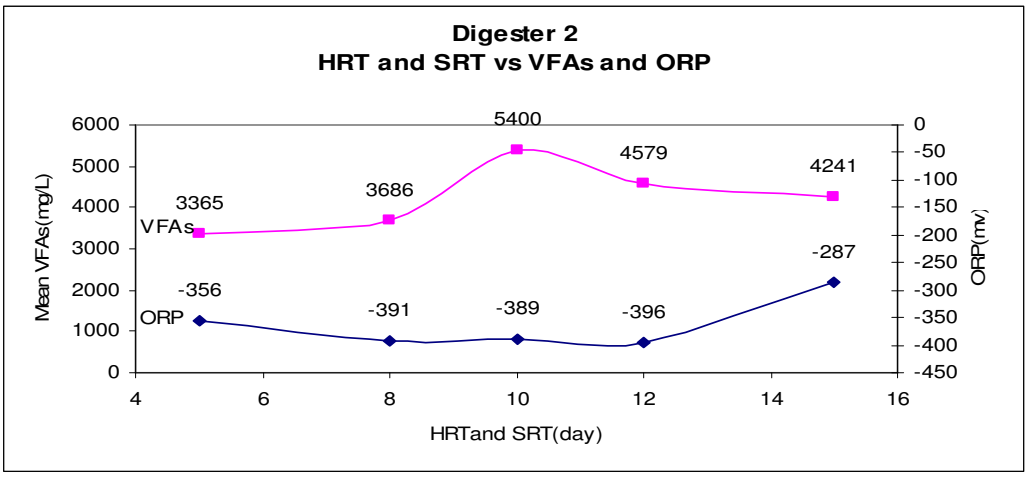


Figure 3.3 VFA and ORP under different SRT conditions in digester 2

The VFAs generated at a 10 day SRT were the highest at 5,400 mg/L, while the lowest value of VFA was 3,365 mg/L at a 5 day SRT. At a 12 day SRT and an 8 day SRT the VFAs were 4,579 mg/L and 3,686 mg/L respectively while 4,241 mg/L of VFA was recorded at a 15 day SRT. An ANOVA test of VFA values for the entire period are shown in Table 3.3 and the results indicate that there were significant differences between VFA values, since the F value (32) is higher than F critical value (2.6) with the probability (9.3×10^{-13}) being very low.

Table 3.3 ANOVA test of VFA values for digester 2

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	27691056	4	6922764	32	9.3E-13	2.6
Within Groups	9956090	46	216436.7			
Total	37647146	50				

The minimum ORP value was -396 mv at a 12 day SRT with a maximum ORP value of -287 mv at a 15 day SRT in digester 2. The ORP values at the 5, 8 and 10 day SRT were -356 mv, -391 mv and -389 mv respectively. The results of an ANOVA test on ORP values at the 8, 10 and 12 SRT are shown in Table 3.4 and they indicate that there was no significant difference between

ORP values.

Table 3.4 ANOVA test of ORP values at an 8, 10 and 12 SRT for digester 2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2206	2	1103	0.064	0.94	3.4
Within Groups	431392.6	25	17255.7			
Total	433598.7	27				

There is no universally suitable SRT for all anaerobic digesters due to varying local conditions; however, in this research an optimum SRT that produced the maximum amount of VFAs could be identified. In this study, VFA generation from both reactors showed maximum VFA production at a 10 day SRT as compared to Lilley et al. (1990) who found an 8 day SRT generated the maximum VFAs.

According to the results from this research, ORP is not a good predictor of the maximum generation of VFAs in an acid-phase anaerobic digester, since the VFA values reached a maximum at a 10 day SRT while the ORP values were highly similar at the 8, 10 and 12 day SRTs in both digesters. The ANOVA test for ORP data showed no significant differences between ORP values in both digesters for the 8, 10 and 12 day SRT but significant differences between VFA values. Thus, ORP can not reveal the exact optimum point for maximum VFA production. The ORP can however (Ⅰ) indicate a range for VFA generation which includes the maximum VFA generation point as well as (Ⅱ) indicate boundary conditions where there is less VFA generation. For example, an ORP range of -315 ± 5 mv in digester 1 indicates good VFA generation. In contrast, if the ORP value increased to higher than approximately -275 mv, VFA generation was much less than the maximum. In digester 2, an ORP range of – 390 ± 5 mv indicated excellent VFA generation. Thus, in this study, ORP values should be maintained below -315 mv to -390 mv in order to reduce the chance of less VFA generation.

Wang et al. (2006) indicated in their study that ORP values dropped gradually to -350 mv as fermentation proceeded in an acid phase anaerobic digester. To maintain ORP values at lower than -315 mv without a huge production of methane, the retention time has to be controlled carefully. Too

long a retention time could cause low ORP but also produce more methane. One possible way to maintain an ORP of -315 mv is using chemical reduction reagents. For example, ammonia (NH_3) or sulfides (H_2S) could be used as reducing agents to decrease the ORP value (Gerardi 2007).

The initial idea behind this research was that VFAs might have a distinct relationship with ORP; however, the results of Figures 3.2 and 3.3 indicate that there was no tight relationship between these two parameters. One of the reasons for the poor correlation between ORP and VFA production could be the characteristics of the ORP measurement. As mentioned ORP is a mixed potential representing the redox-potential level of all the biochemical reactions in the system. As will be seen shortly, VFA concentrations in both digesters were less than 50 % of the SCOD which means that other substrates were produced, most probably lactic acid and alcohols. Differing amounts of these substrates may affect the ORP value. The percent of VFA in SCOD is shown in Table 3.5 and depicted in Figure 3.4.

Table 3.5 VFA percent in SCOD in digester 1 and 2

Digester1				Digester2			
HRT/SRT	VFA	SCOD	VFA % in SCOD	HRT/SRT	VFA	SCOD	VFA % in SCOD
5	3645	11936	30.5	5	3365	11982	28.1
8	3878	13008	29.8	8	3686	12408	29.7
10	5556	13691	40.6	10	5400	14005	38.6
12	4606	13269	34.7	12	4579	14150	32.4
15	4385	13261	33.1	15	4241	13244	32.0

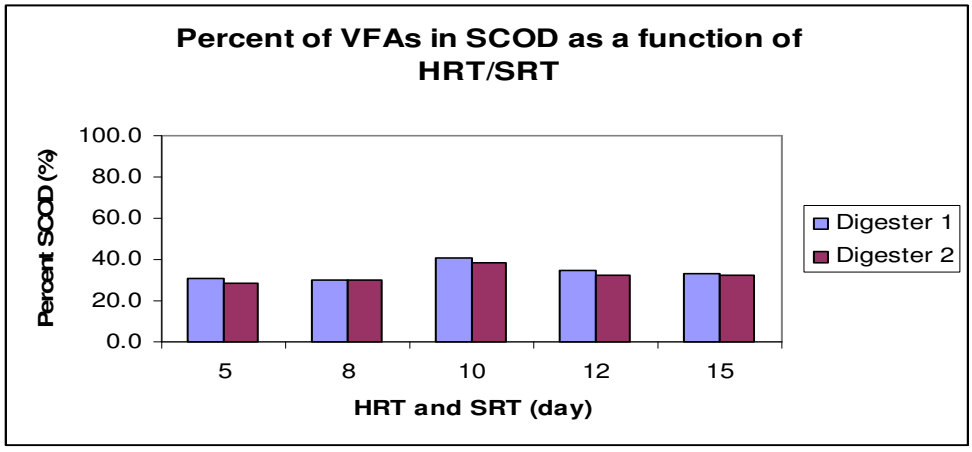


Figure 3.4 VFA (%) in SCOD as a function of HRT/SRT in digester 1 and 2

3.2 Other parameters investigated while adjusting SRT

The raw data for alkalinity, VSS, SCOD, pH and temperature for digester 1 and 2 for the entire experimental period are shown in Figures 3.5 and 3.6. Again the initial 5 data points when an SRT condition was changed were ignored to allow the system to settle down.

The VSS, SCOD and alkalinity values for digester 1 as a function of SRT are shown in Figure 3.7. Particulate matter in sludge must first be hydrolyzed or liquefied before being assimilated by bacteria, resulting in a reduction in VSS concentration (Maharaj and Elefsiniotis 2001); thus, again the condition of a 10 day SRT was most preferable for anaerobic bacteria because of the large reduction in VSS as well as high SCOD production. The VSS decreased from 26,879 mg/L to 24,153 mg/L (10.1% reduction) at a 5 day SRT and to 13,691 mg/L (49.0% reduction) at a 10 day SRT. The VSS also decreased to 21,975 mg/L (18.2% reduction) at a 15 day SRT. As can been seen then, the most VSS reduction occurred at a 10 day SRT. The ability of a bacterial population to convert large, insoluble macromolecules into monomeric, soluble molecules (alternately called hydrolysis, solubilization, or liquefaction) can also be measured through SCOD values (Elefsiniotis et al. 2005). The SCOD increased from an inflow value of 8,676 mg/L to 11,936 mg/L (37.6 % increase) at a 5 day SRT and to 13,691 mg/L (57.8 % increase) at a 10 day SRT. The SCOD also increased to 13,261 mg/L (52.8 % increase) at a 15 day SRT. Thus, the most SCOD production was also at a 10 day SRT.

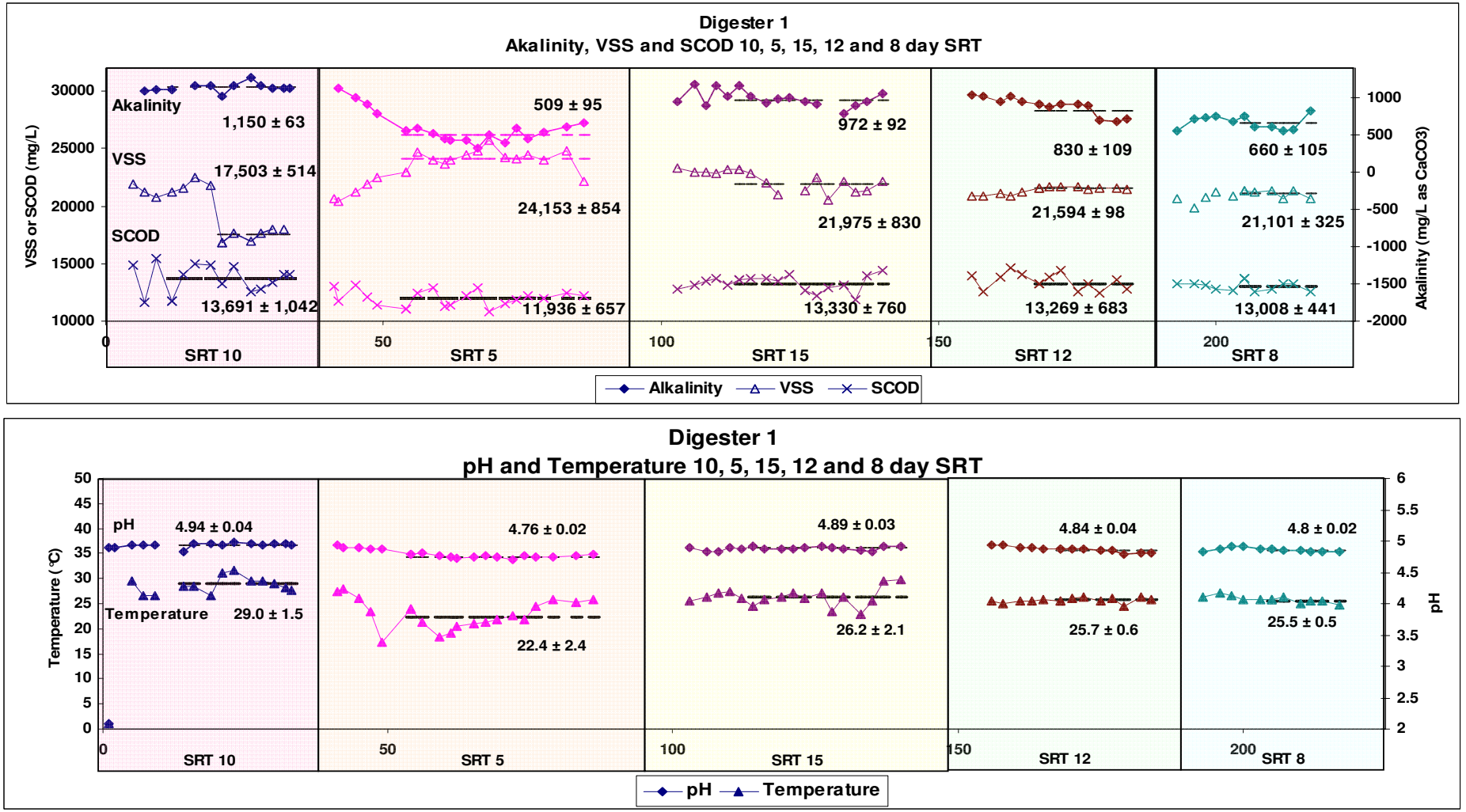


Figure 3.5 Other parameters investigated in digester 1 as a function of SRT for the entire experiment period

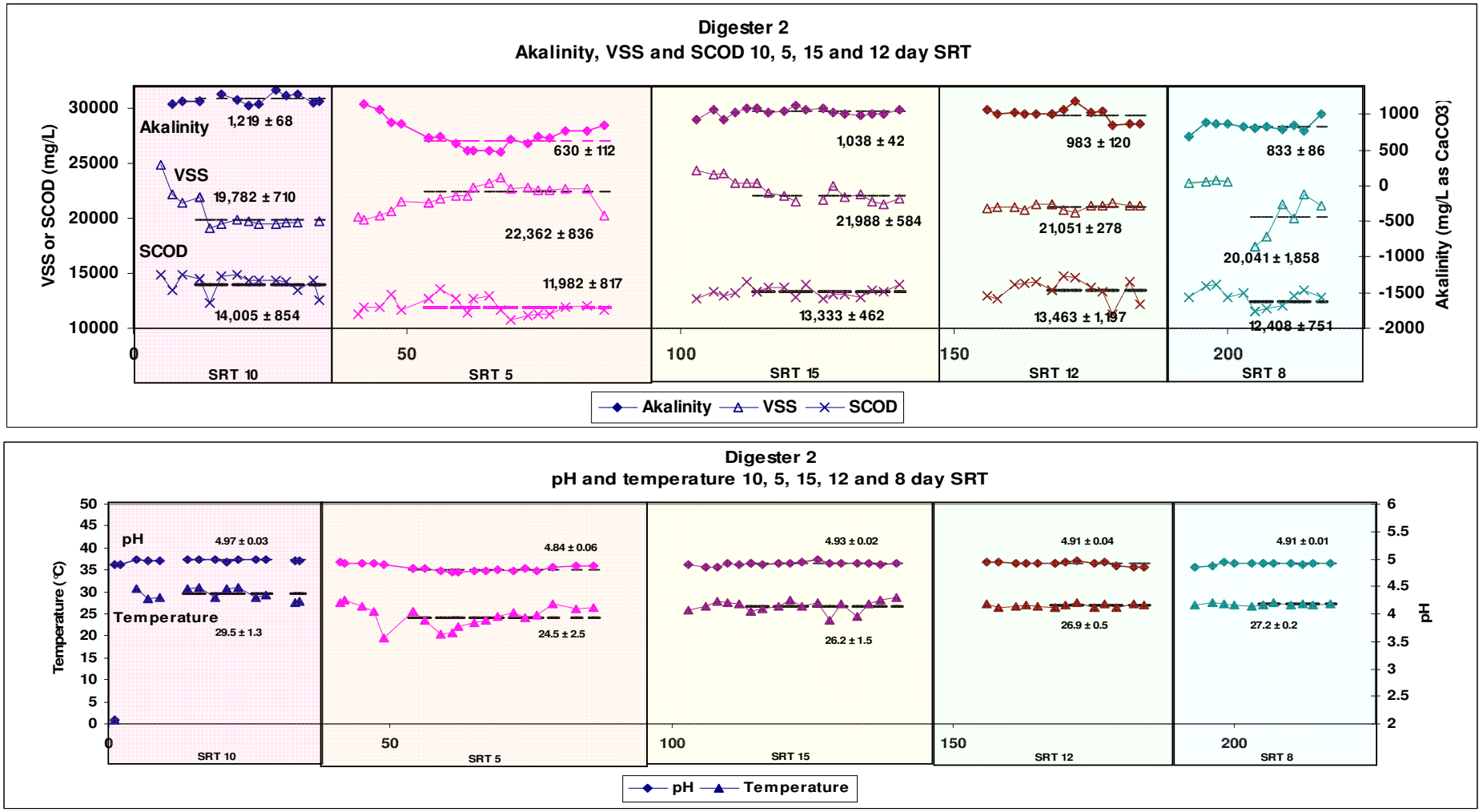


Figure 3.6 Other parameters investigated in digester 2 as a function of SRT for the entire experiment period

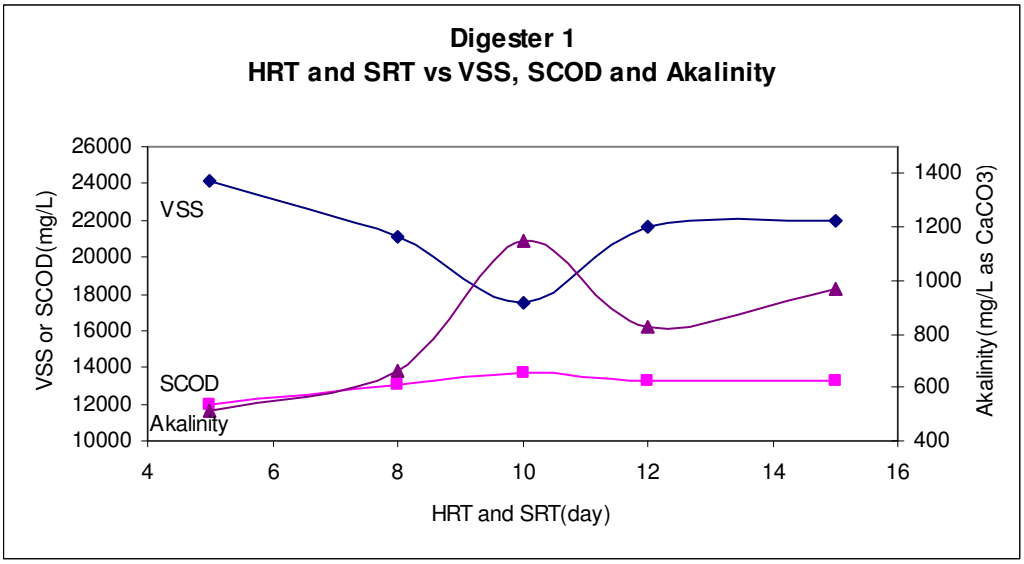


Figure 3.7 VSS, SCOD and alkalinity under different SRTs in digester 1

Alkalinity values as a function of the different SRTs in digester 1 are shown in Figure 3.8 which indicates that there was a good linear relationship between SRT and alkalinity. Alkalinity increased gradually from 509 mg/L at a 5 day SRT to 972 mg/L at a 15 day SRT (ignoring momentarily the data for the 10 day SRT). Theoretically, the alkalinity at a 10 day SRT should have the lowest value since the most VFA were produced; however, the alkalinity was the highest value at 1,150 mg/L. This may be explained by the presence of ammonia and bicarbonate since it is generally accepted that the formation of NH_4HCO_3 can increase the total alkalinity (Alvarez 2003).

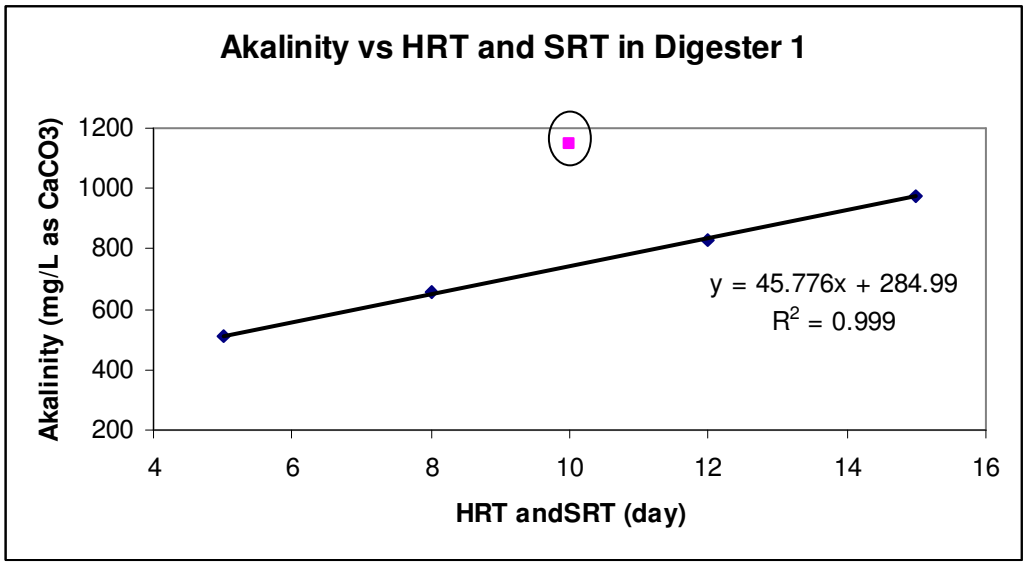


Figure 3.8 Alkalinity with different SRTs in digester 1

That is, the total alkalinity could be enhanced by ammonia coming from protein which is 35 – 40 % of the feed source in our study. Proteins are hydrolyzed and converted into individual amino acids during acidification. The most common pathway for the anaerobic digestion of amino acids is deamination and the subsequent conversion to the corresponding VFA (Fox and Pohland 1994). An example is the deamination of leucine:

$$\text{Leucine} + 3H_2O \rightarrow \text{Isovalerate} + HCO_3^- + H^+ + NH_4^+ + 2H_2$$

The concentration of valeric acid as a function of SRT is shown in Figure 3.9 and it can be seen that there was 600 – 1500 mg/L of valeric acid measured in both digesters. As it clear that the most valeric acid was produced during the SRT of 10 days and this may be the reason for the large alkalinity.

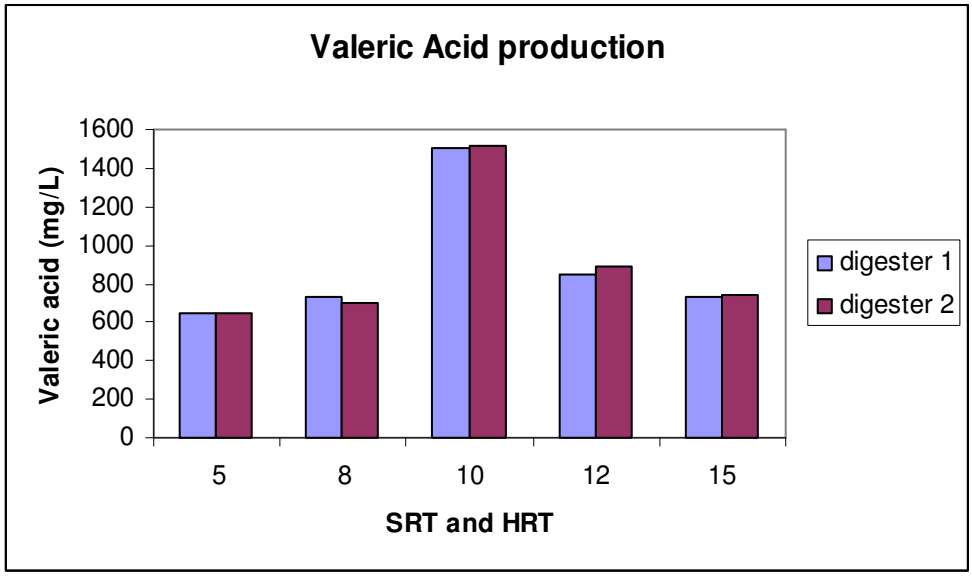


Figure 3.9 Valeric acid production in digester 1 and 2 as a function of SRT

Metcalf and Eddy (2003) argued that VFAs may not be the principal consumer of alkalinity in a digester. They indicated that the principal consumer of alkalinity in a digester is carbon dioxide, and not volatile fatty acids as is commonly believed. That is, due to the partial pressure of gas in a digester, the carbon dioxide goes into solution and forms carbonic acid, which consumes alkalinity (Metcalf and Eddy 2003). The reactions to consume alkalinity are:

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$
$$H_2CO_3 + OH^- \leftrightarrow HCO_3^- + H_2O$$
$$HCO_3^- + OH^- \leftrightarrow CO_3^- + H_2O$$

The VSS, SCOD and alkalinity values for digester 2 as a function of SRT are shown in Figure 3.10. Trends for Figure 3.10 are similar to digester 1.

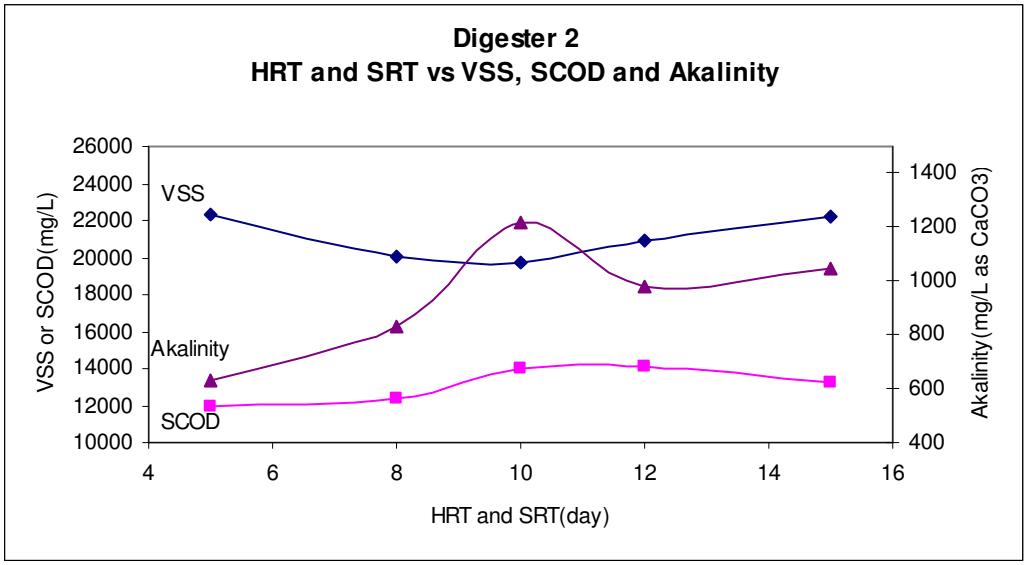


Figure 3.10 VSS, SCOD and alkalinity under different SRTs in digester 2

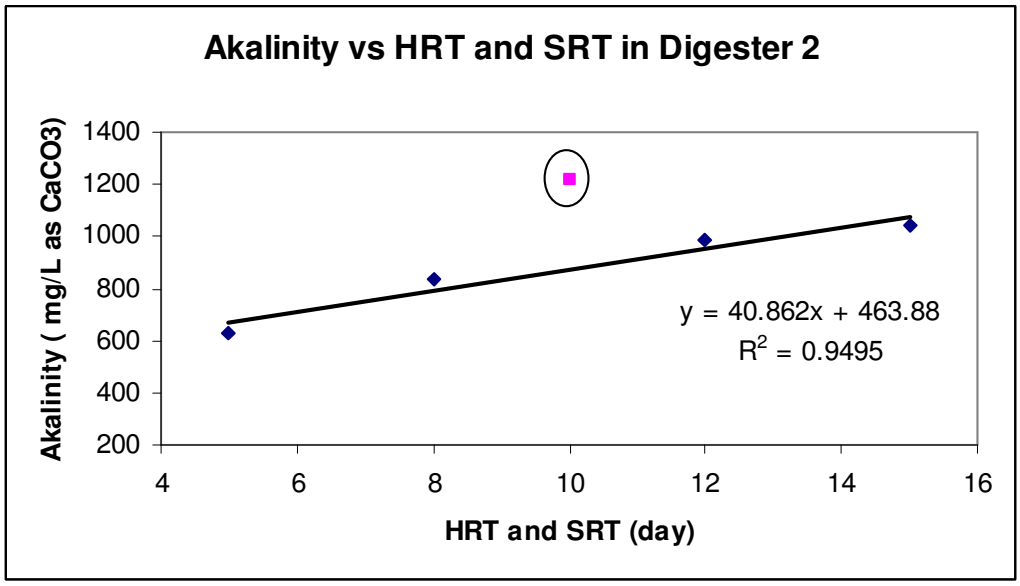


Figure 3.11 Alkalinity with different SRTs in digester 2

The temperature and pH profiles for digester 1 and 2 are shown in Figures 3.12 and 3.13. For digester 1, the temperature was the highest value at a 10 day SRT (29.0 ℃) and the lowest value at a 5 day SRT (22.4 ℃) while the pH was stable between 4.8 and 4.9. The highest temperature was observed at a 10 day SRT which correlates to the highest VFA, SCOD and VSS reduction (i.e. more biological reactions).

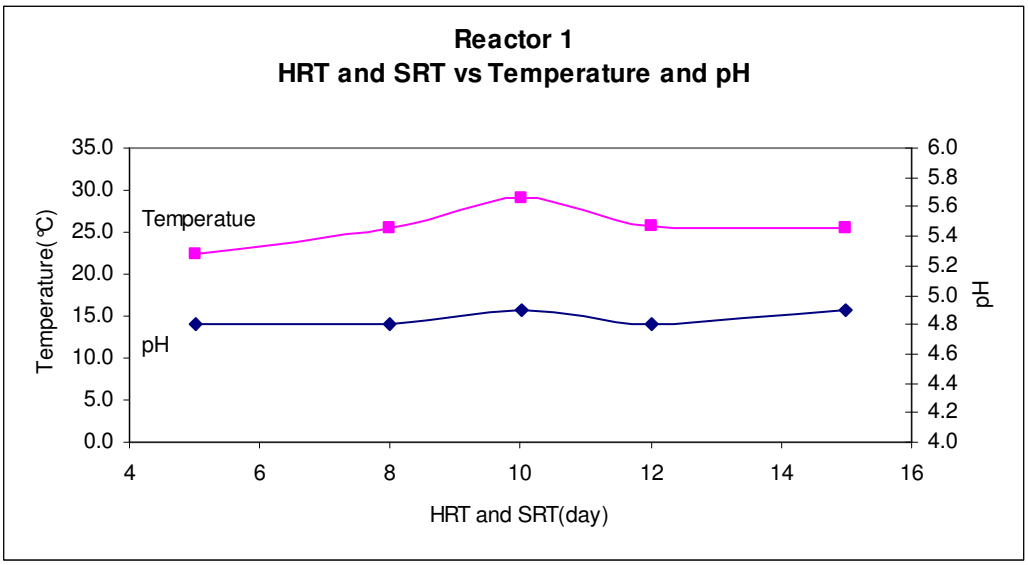


Figure 3.12 Temperature and pH under different SRTs in digester 1

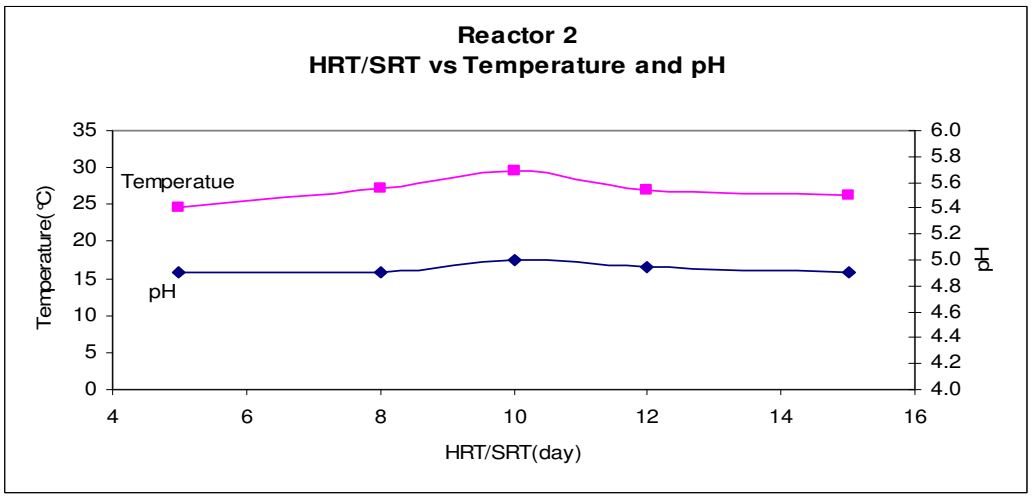


Figure 3.13 Temperature and pH under different SRTs in digester 2

3.3 Combining other parameters with ORP to predict VFAs

As mentioned previously, the results of this study indicate that ORP by itself is not a good predictor of maximum VFA production. Akin and Ugurlu (2005) indicated that ORP cannot be a reliable control parameter on its own,

however with pH; ORP could explain biological nutrient removal reactions. Additionally, Zoetemeyer et al. (1982) stated that the products of acid fermentation were dependent on the culture pH value; thus, by selecting an appropriate pH value the major products could be predicted. In this study the pH was very stable at 4.8 ± 1 in both reactors across all SRTs meaning that pH would not be useful in combining with ORP to predict maximum VFA generation. In essence, the pH was just a parameter in the manner of Elefsiniotis et al. (2005) who indicated that pH is an important reflection of anaerobic digestion stability. In their study, pH fluctuations ranged from 2.4 to 5.8 % of the mean value, which indicated a reasonably stable system.

Alkalinity may be an alternate parameter which can be combined with ORP to predict optimum VFA production. Anderson and Yang (1992) indicated that the total alkalinity value includes all the bicarbonate, and approximately 80 % of the VFA. However, because only bicarbonate is usable for neutralizing VFAs, total alkalinity dose not always represent the available buffering capacity in a digester. This disadvantage has limited the use of alkalinity by itself as a useful parameter for monitoring dynamic behavior of a digester (Anderson and Yang 1992). Relationships between alkalinity and VFA in this study are shown in Figures 3.14 and 3.15. From the Figures, the alkalinity has a good linear relationship with VFA production in both digesters (R^2 = 86.6 % and 89.2 % respectively).

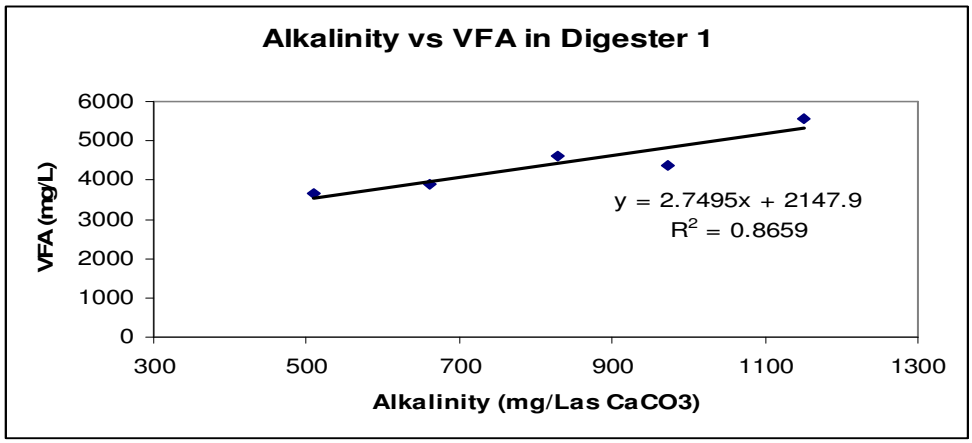


Figure 3.14 Relationship between VFA and alkalinity in digester 1

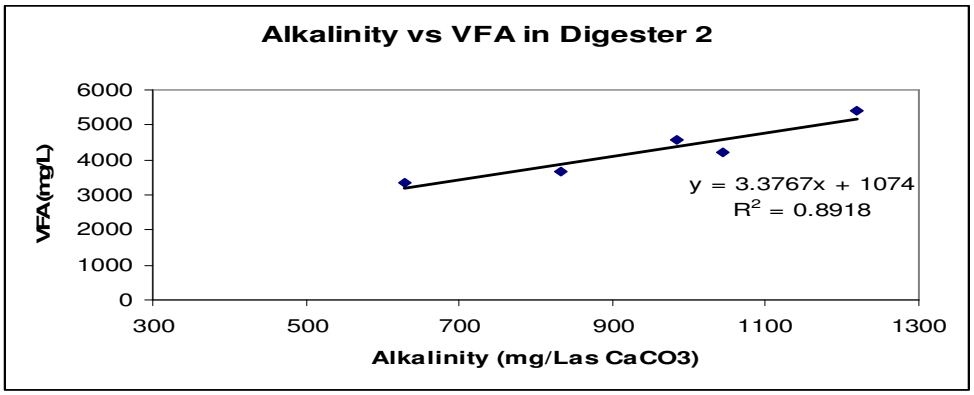


Figure 3.15 Relationship between VFA and alkalinity in digester 2

A relationship between VFAs and ORP combined with temperature was also sought and the following equation in Figure 3.16 was derived from the measured data using multiple regression analysis. Li and Bishop (2004) also indicated that temperature can indirectly affect ORP values and by combining ORP with temperature, effluent quality can be predicted accurately. According to the results in Figure 3.16, the overall equation is significant because the P-value of F-statistics is 0.0042 (less than 0.05) and the adjusted R-squared is sufficiently high at 0.73 indicating that the equation fits the data well. There is only a 5 % chance that results could have surfaced in a random distribution with a P of 0.05. Thus, it can be said that with a P value of 0.0042, the equation is specified correctly.

In the equation every unit increase in ORP leads to a 7.05 mg/L increase in VFAs (with everything else constant). Furthermore, every degree increase in temperature increases VFAs by 387.29 mg/L (with everything else constant). Thus, the temperature effect on VFA production is significantly more than the effect of an ORP change. The coefficients for ORP and intercept are only significant at 10 % level (but not at the 5% level) since the P value is higher than 0.05. However, the coefficient of temperature is significant at the 5 % level, since the P-value of temperature is less than 0.05. In contrast, the t statistic values for all coefficients of independent variables are significant, since the absolute values of the t statistic are higher than 2. The t-statistic is a measure of how strongly a particular independent variable explains variations in the dependent variable and a t-statistic of 2 is generally accepted as being of statistical significance. Therefore, ORP and temperature both affect VFA production but temperature affects it more significantly.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.89
R Square	0.79
Adjusted R Square	0.73
Standard Error	379.69
Observations	10.00

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	2.00	3807281.71	1903640.86	13.20	0.0042
Residual	7.00	1009139.19	144162.74		
Total	9.00	4816420.90			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-3502.45	1618.03	-2.16	0.067	-7328.48	323.58	-7328.48	323.58
ORP	7.05	3.08	2.29	0.056	-0.23	14.34	-0.23	14.34
Temperature	387.29	76.76	5.05	0.002	205.80	568.79	205.80	568.79

VFA = 7.05 ORP + 387.29 T – 3502.45

Adjusted R squared = 0.73

F-statistics (p=0.0042)

Figure 3.16 Equation derived by results through multiple regression analysis

The predicted VFA concentration based on the equations were in good agreement with the measured VFAs with an R^2 = 79.05 % in Figure 3.17.

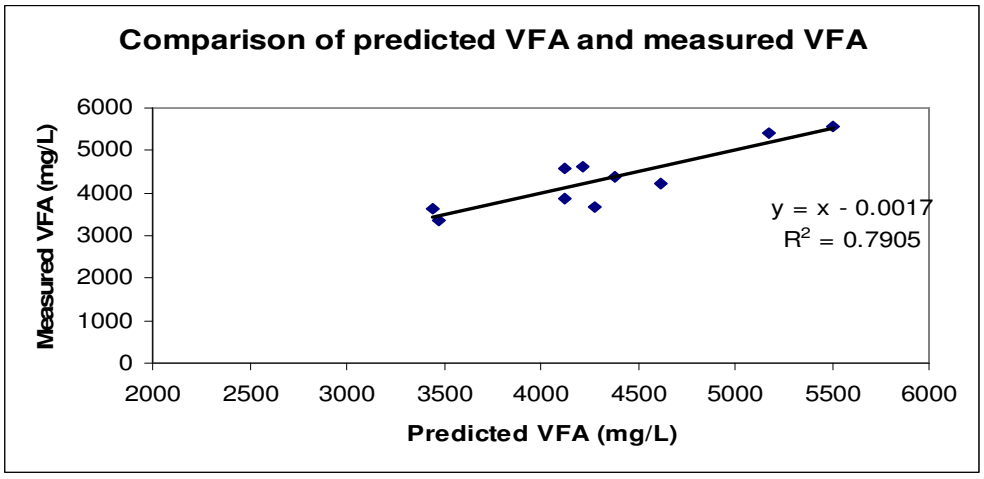


Figure 3.17 Comparison of predicted VFA and measured VFA

3.4 Specific VFA production rate

Tables 3.6 and Table 3.7 show the net VFA production and specific VFA production rates as a function of SRT in digesters 1 and 2. The specific VFA production rate is expressed as the net amount of VFAs generated per day per unit mass of VSS in the reactor (mgVFA/mgVSS*day) and this expression can be found in several other studies (Banerjee et al. 1999; Maharaj and Elefsiniotis 2001; Elefsiniotis et al. 2005).

Table 3.6 Net VFA production and specific VFA production rate as a function of SRT in digester 1

Digester1			
HRT/SRT	Net VFA (mg/L as acetic acid)	VSS (mg/L)	Specific VFA production Rate (mgVFA/mgVSS*day)
5	3645	24153	0.030183
8	3878	21101	0.022973
10	5556	17503	0.031743
12	4606	21661	0.017720
15	4385	21975	0.013303

Table 3.7 Net VFA production and specific VFA production rate as a function of SRT in digester 2

Digester2			
HRT/SRT	Net VFA (mg/L as acetic acid)	VSS (mg/L)	Specific VFA production Rate (mgVFA/mgVSS*day)
5	3365	22362	0.030096
8	3686	20041	0.022990
10	5400	19782	0.027298
12	4579	20896	0.018261
15	4241	22174	0.012751

The specific VFA production rate decreased from 0.030 mgVFA/mgVSS*day at a 5 SRT to 0.013 mgVFA/mgVSS*day at a 15 day SRT in digester 1 and 2. In general, there was good linear relationship in both digesters between the specific VFA production rate and SRT (Figures 3.18 and 3.19) provided the VFA production rates at the 10 day SRT are omitted. This is because the optimum SRT for both digesters was 10 days associated with the generation of high VFAs and significant VSS reduction which means that specific VFA production rates in both digesters were too high to fit in the line in the graphs. In this study, different values of specific VFA production rates were obtained with different SRTs while Elefsiniotis et al. (2005) indicated that the specific VFA production rate was similar between industrial

and municipal mixtures at approximately 0.070 mgVFA/mg VSS*day. A nominal SRT of 10 days was maintained in their study, however their rate was significantly higher than the 10 day SRT result (0.030 mgVFA/mg VSS*day) obtained in this study.

One possible reason may be that the digesters were overloaded on a solids loading basis. The solids loading rate (2.69 kgVSS/m^3*day) for this study fell into the range recommended (1.6 – 4.8 kgVSS/m^3*day, Tchobanoglous et al. 2003) however this was because of the large digesters (20 L) and long HRT (10 day) compared to the 1.5 - 3 L volume of digester and 18 - 30 hr HRT in other studies (Banerjee et al. 1999; Elefsiniotis et al. 2005). That is both the volume and HRT minimized the influent VSS concentration of 26,879 mg/L which was very high compared to the approximately 4,000 mg/L for Banerjee et al.'s study (1999). Elefsiniotis et al. (2005) also maintained on influent VSS concentration of approximately 4,700 mg/L in their study. Metcalf and Eddy (2003) recommended 1 - 3 day SRT in acid-phase anaerobic digesters with 50 to 60 % of volatile solids reductions and as seen the SRTs in this study were consistently longer than these values.

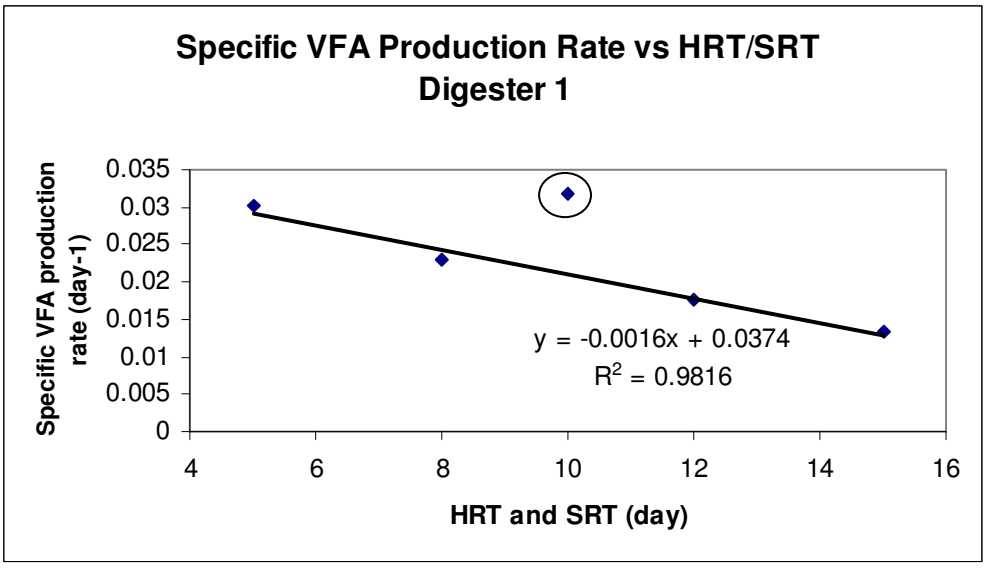


Figure 3.18 Relationship between specific VFA production rate and SRT in digester 1

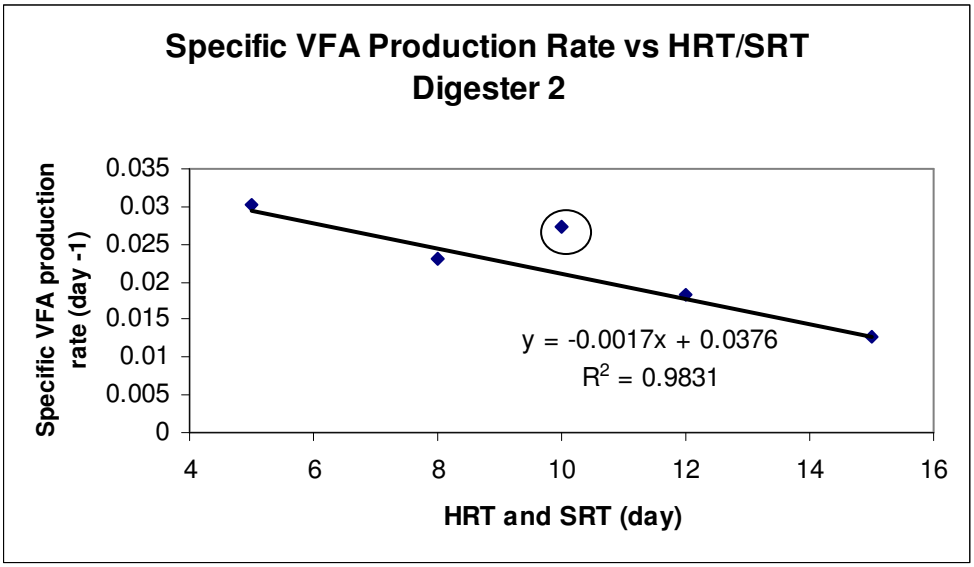


Figure 3.19 Relationship between specific VFA production rate and SRT in digester 2

3.5 Gas production rate

One of the indications of successful accomplishment of acid-phase anaerobic digester is a low gas production. Figure 3.20 and Figure 3.21 show average gas production (from intermittent spot checks) and the values were 178.2 mL/hr (4.3 L/day) and 132.2 mL/hr (3.2 L/day) at the 10 day and the 5 day SRT, respectively.

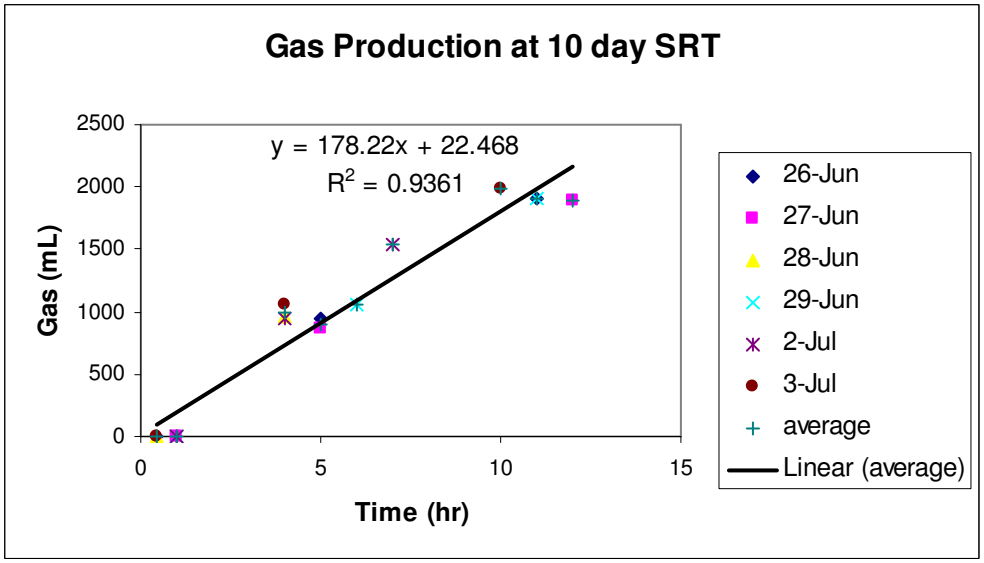


Figure 3.20 Gas production rate at a 10 day SRT

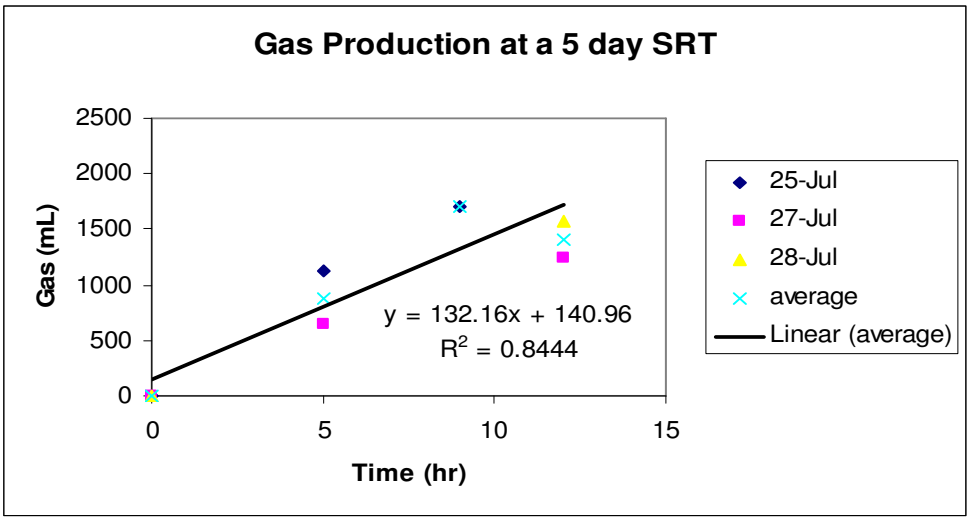


Figure 3.21 Gas production rate at a 5 day SRT

The gas composition and production rate at the 5 and 10 day SRT are shown in Table 3.8 and 3.9. The gas composition was an average of 19 % CH_4, 76 % CO_2, and 5 % others (i.e. H_2, H_2S, and N_2). The hydrogen production was very little in this study since all the other gases including hydrogen were only 6.5 % in digester 1 and 2.9 % in digester 2 respectively. Shana et al. (1996) indicated that the gas composition averaged 22 % CH_4 and 74 % CO_2 in their study. In another study, 32 % CH_4 and 62 % CO_2 were calculated for all runs (Elefsiniotis et al. 1996). The gas production rate at a 10 day SRT was higher than at a 5 day SRT which indicates that at the shorter SRTs, the carbon compounds were being kept in the acid phase.

Table 3.8 Gas production at 10 day SRT

10 day SRT			
	Concentration (%)	Gas production rate (L/day)	Gas production (m^3/kg VSS destroyed)
CH_4	19.1	0.8	0.0695
CO_2	74.7	3.2	0.2717
Others	6.5	0.3	0.0238
Total		4.3	0.365

Table 3.9 Gas production at 5 day SRT

5 day SRT			
	Concentration (%)	Gas production rate (L/day)	Gas production (m^3/kg VSS destroyed)
CH_4	19.8	0.6	0.0473
CO_2	77.3	2.4	0.1824
Others	2.9	0.1	0.0053
Total		3.2	0.235

Theoretically, methane should be negligible in an acid-phase anaerobic digester because of phase separation; however, around 19 % of the gas was methane in both digesters, evidencing that there were some methanogenic activity. Other researchers, Elefsiniotis et al. (1996) deduced that production of a certain amount of methane is due to either incomplete separation of the two phases or the presence of certain fast-growing autotrophic methanogens such as Methanobacterium.

The gas production rates were 0.365 m^3/kg VSS destroyed at a 10 day SRT and 0.235 m^3/kg VSS destroyed at a 5 day SRT. The gas production for acid-phase anaerobic digesters ranged between 0.15 – 2 m^3/kg VS destroyed in Shana et al.'s (1996) study therefore values from this study were just inside that range.

3.6 Relationship between ORP and VFAs and other parameters as a function of temperature

The raw data for VFAs and ORP for digester 1 and 2 as a function of temperature (8, 29 and 37℃) for the entire experimental period are shown in Figure 3.22. All data is for a 10 day SRT. As expected, VFA production changed with temperatures since VFA producing bacteria are affected by temperature (Cha and Noike 1997).

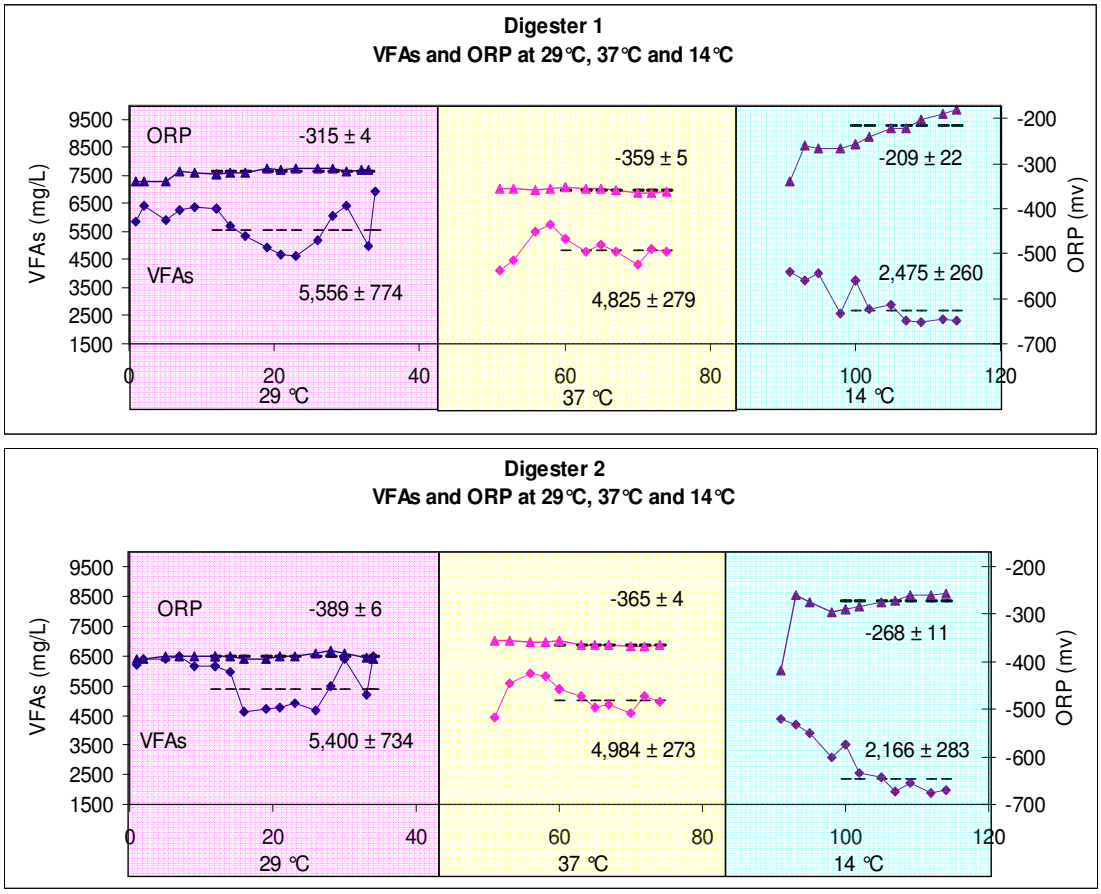


Figure 3.22 VFAs and ORP in digester 1 and 2 as a function of temperature for the entire experimental period

Figures 3.23 and 3.24 show the mean VFA production and ORP variation at different temperatures and they indicate that a maximum VFA value of 5,556 mg/L was produced at 29 ℃ in digester 1. VFAs of 2,475 mg/L and 4,825 mg/L were generated at 14 ℃ and 37 ℃ respectively. In digester 2, VFAs of 5,400 mg/L were produced at 29 ℃ and 2,166 mg/L and 4,984 mg/L were generated at 14 ℃ and 37 ℃ respectively.

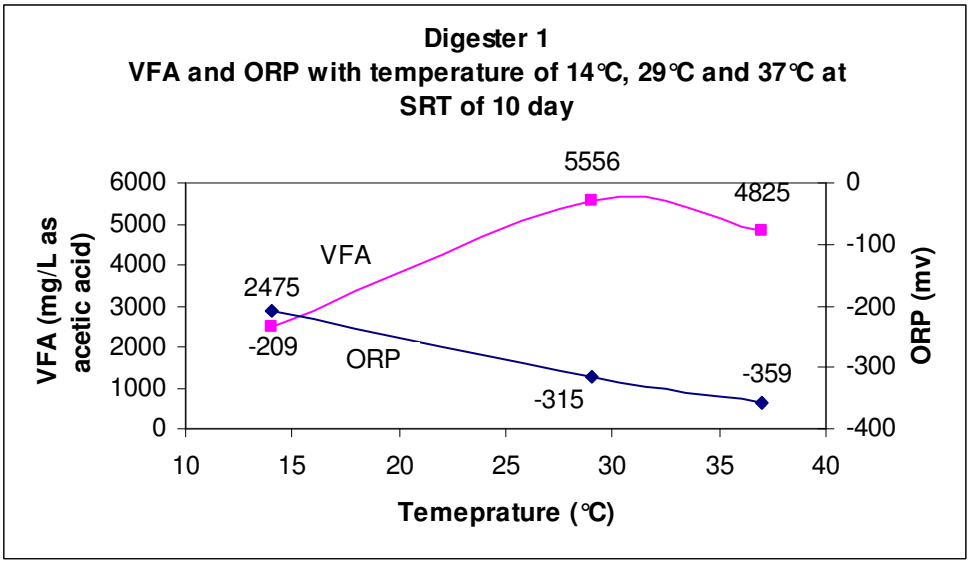


Figure 3.23 Variation in VFA production and ORP with temperature at SRT of 10 days in digester 1

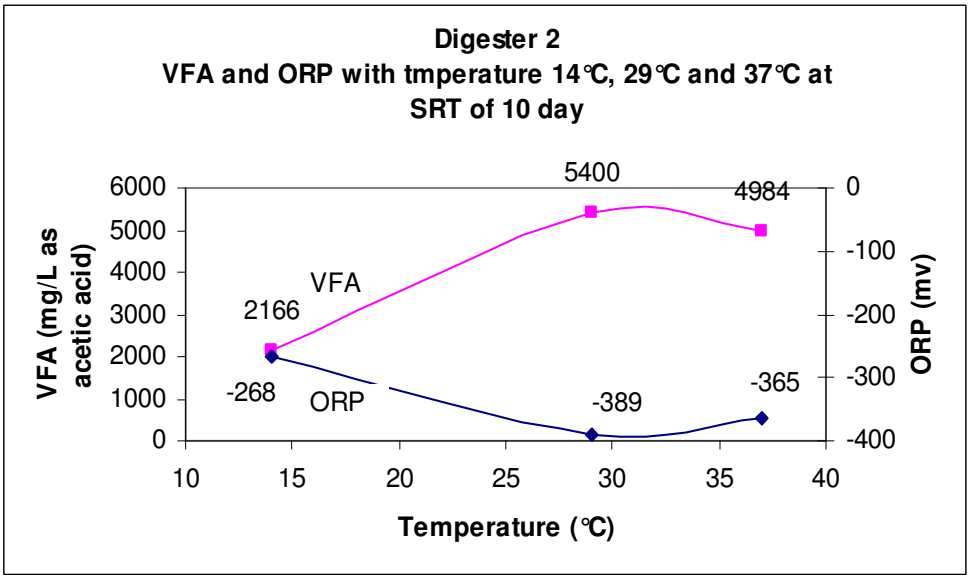


Figure 3.24 Variation in VFA production and ORP with temperature at SRT of 10 days in digester 2

It can be seen that a temperature of 29 ℃ was the optimum for both digesters as it produced the maximum amount of VFAs. Banerjee et al. (1999) indicated that VFA production went up by 15 % when the temperature was increased to 30 ℃, but it decreased by 23 % at 35 ℃. The optimum temperature may however depend upon the type of bacteria growing in the digester and the substrate being consumed.

The ORP values were -209 mv at 14 ℃, -315 mv at 29 ℃ and -359 mv at 37 ℃ in digester 1 while, in digester 2, they were -268 mv at 14 ℃, -389

mv at 29 ℃ and -365 mv at 37 ℃. The most negative ORP values were recorded at 37 ℃ in digester 1 and at 29 ℃ in digester 2. Since the VFA production at 37 ℃ was approximately 87 % of the maximum VFAs at 29 ℃, this could indicate that 37 ℃ was not an extreme condition for VFA generating bacteria. It was however noted that high ORP values correlated with low VFA production at 14 ℃ in both digesters.

The raw data for alkalinity, VSS, SCOD, pH and temperature for digester 1 and 2 for the entire experimental period at the different temperatures are shown in Figure 3.25 and 3.26.

Figures 3.27 and 3.28 show the mean values of VSS, SCOD and alkalinity in digester 1 and 2 as a function of temperature. Although the maximum VSS reduction should theoretically correlate to maximum SCOD production, the maximum VSS reduction occurred at 29 ℃ in both digesters, while the highest SCOD values were measured at 37 ℃ in both digesters. It is suspected that the temperature of 29 ℃ and 37 ℃ were not significantly different enough to affect VSS reduction and SCOD production. In contrast, the SCOD values at 14 ℃ were measured a 12,267 mg/L and 11,150 mg/L (digester 1 and 2 respectively) and these values were the lowest values in the temperature range investigated in this study.

The values of alkalinity were similar at 29 ℃ and 37 ℃ in both digesters. Namely, the alkalinity was 1,150 mg/L at 29 ℃ for digester 1 and 1,218 mg/L for digester 2 while they were 1,289 mg/L for digester 1 and 1,187 mg/L for digester 2 at 37 ℃. In contrast, the alkalinity values at 14 ℃ were 0 in both digesters, since the pH fell below 4.5. As shown in Figure 3.29 and 3.30, the pH of both digesters was approximately 4.1 at 14 ℃ which means that all the buffer capacity was used by the VFAs produced, even though the amount of VFAs produced was very small. The concentrations of ammonia were 344 mg/L and 382 mg/L at 29 ℃ dropping to 159 mg/L and 137 mg/L at 14 ℃ in digester 1 and 2 respectively. The alkalinity at 14 ℃ (likely arising from ammonia) may be insufficient for anaerobic microbial activity to compensate for the acidity produced by acidogens.

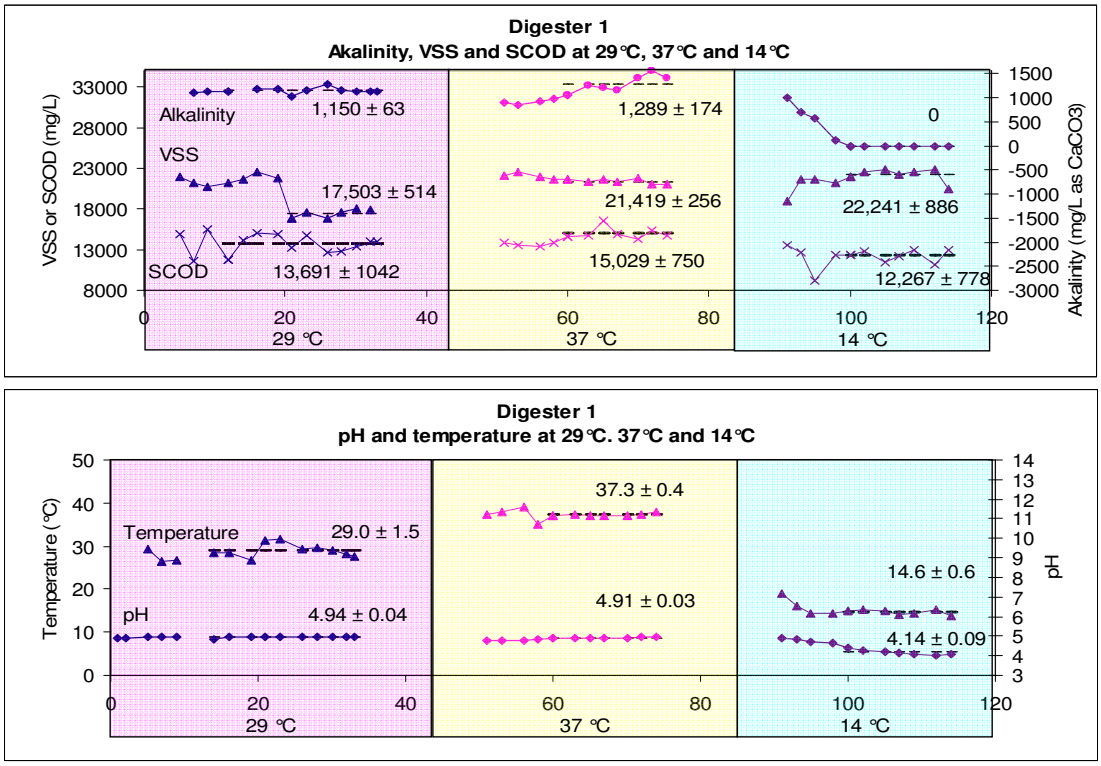


Figure 3.25 Other parameters for digester 1 as a function of temperature (SRT=10 days)

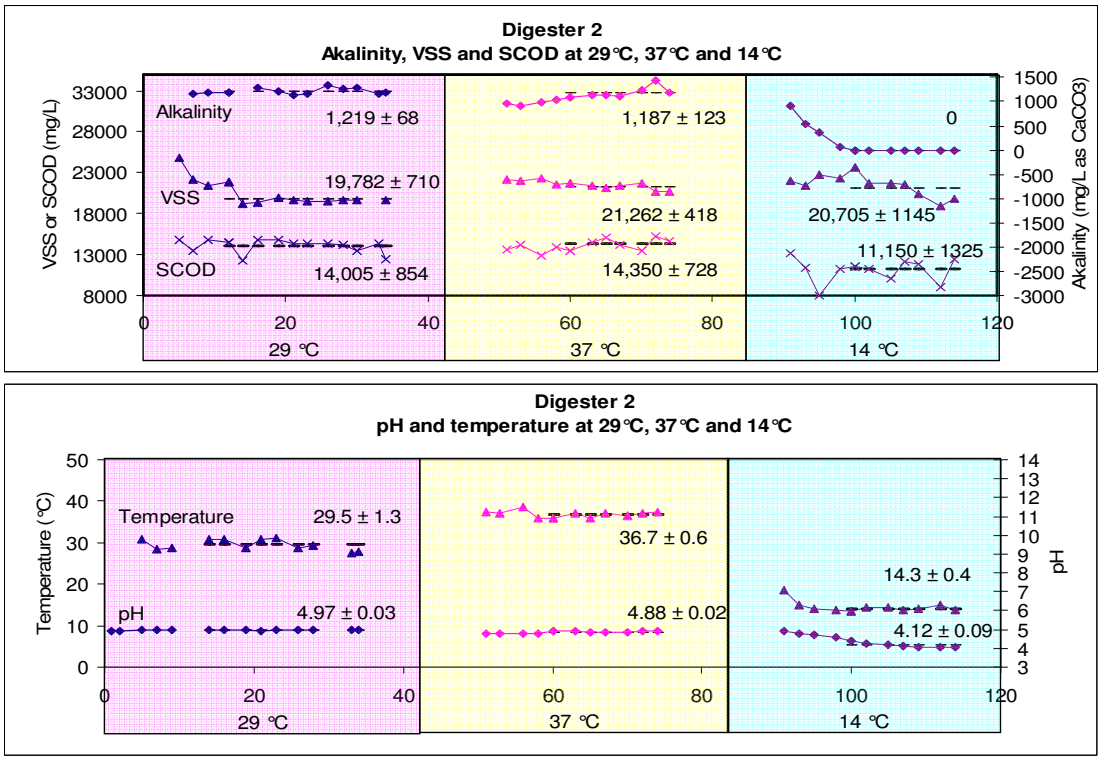


Figure 3.26 Other parameters for digester 2 as a function of temperature (SRT=10 days)

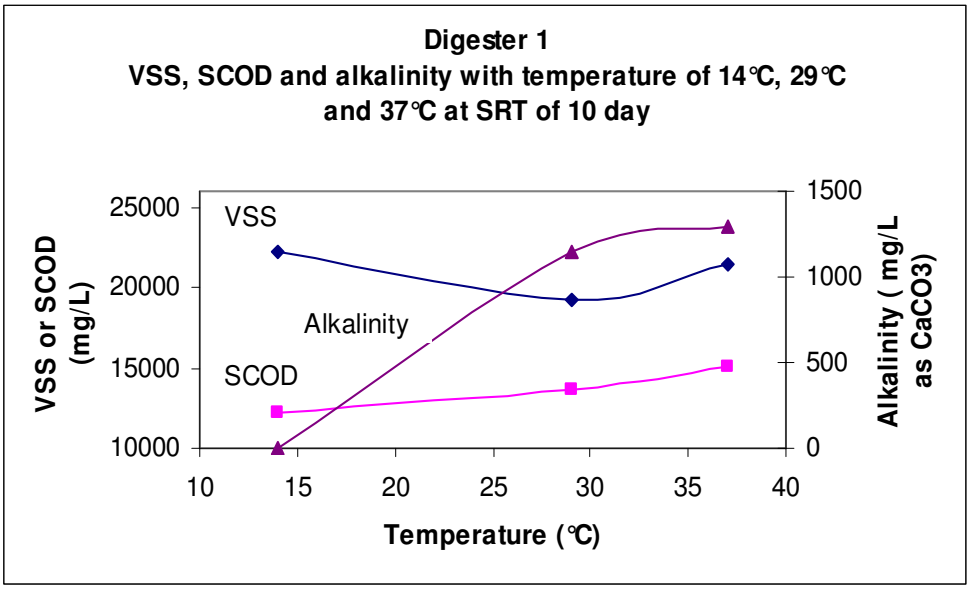


Figure 3.27 VSS, SCOD and alkalinity under different temperatures in digester 1

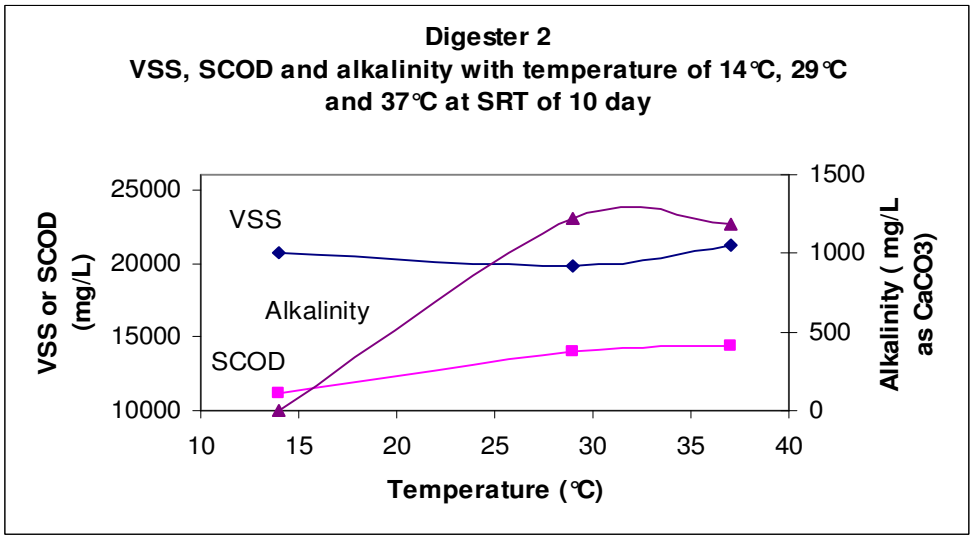


Figure 3.28 VSS, SCOD and alkalinity under different temperatures in digester 2

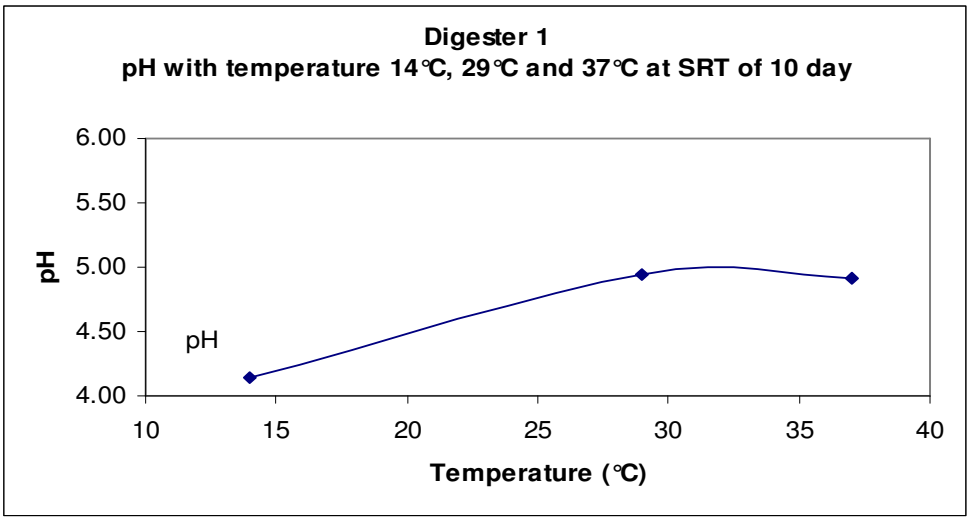


Figure 3.29 pH under different temperatures in digester 1

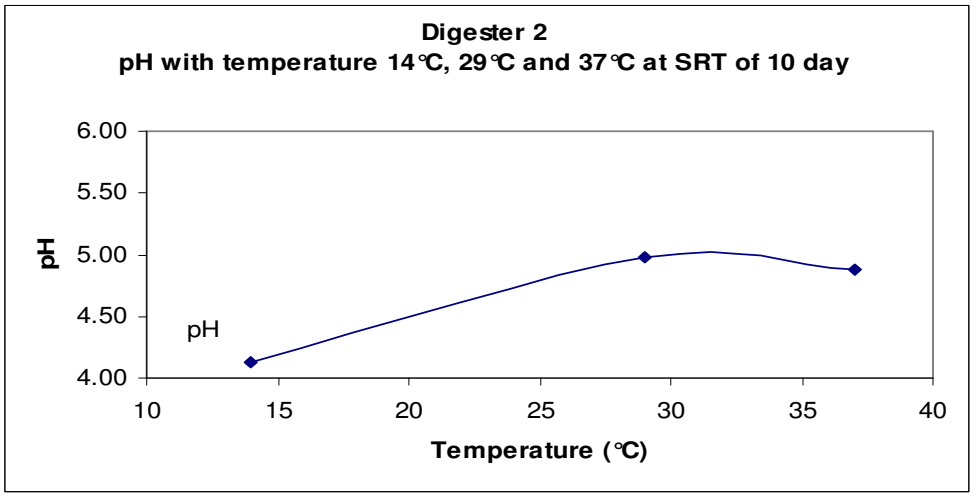


Figure 3.30 pH under different temperatures in digester 2

It was observed that a significant amount of non-VFA substrate was produced at 14 ℃ in both digesters. Figures 3.31 and 3.32 confirm that this substrate was ethanol. The graph was generated by using the gas chromatograph retention time results as well as the boiling points of the substrates investigated. There was a very good linear relationship between retention time and boiling point, thus, the substrate with a retention time at 1.620 minutes was confirmed as being a substrate with a boiling point of 78 ℃ (i.e. ethanol). Ethanol is the product of anaerobic biological processes by which sugars such as glucose, fructose, and sucrose, are converted into ethanol and carbon dioxide. Zoetemeyer et al. (1982) also indicated that ethanol can be produced at low temperatures in an anaerobic digester.

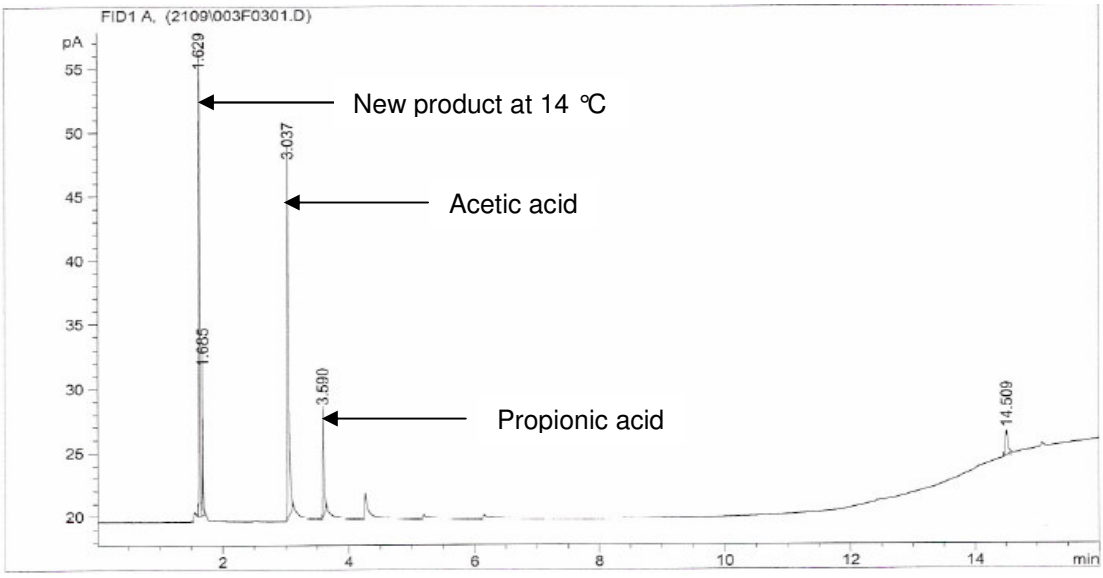


Figure 3.31 Typical gas chromatograph results at 14 ℃

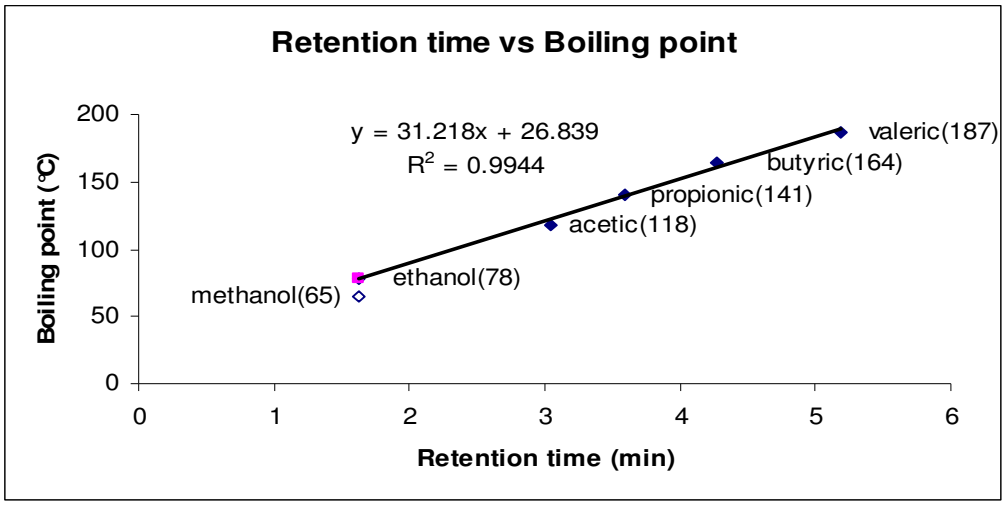


Figure 3.32 The relationship between retention time of a gas chromatograph and the boiling point of VFAs

In summary, VFA production was reduced at the low temperature while ORP, VSS, SCOD and alkalinity were also affected. However, more than 2,000 mg/L VFA was still found at 14 ℃ in both digesters.

4 Conclusions

1. There was no tight relationship between VFA production and ORP while adjusting SRT in an acid-phase anaerobic digestion process. This is because the ORP measurement in anaerobic digestion is the net value of oxidation and reduction caused by many factors, such as the electrode itself, the concentration of reactants and products as well as the bacterial activity.

2. ORP may indicate a range in which the operator should strive to avoid boundary conditions that impair VFA generation. According to the results of this study, an ORP range of approximately – 310 to -390 mv should be maintained to maximize VFA production. This range seems to suppress the activity of methane forming bacteria.

3. ORP by itself can not predict maximum VFA generation. However, ORP could be combined with temperature to show a linear relationship with respect to VFA generation. The equation below was developed for this study.

   VFA = 7.05 ORP + 387.29 T – 3502.45

4. In this study, minimal VFA production with high ORP values was measured at 14 ℃ (as compared to 29 ℃ and 37 ℃). However, there was no significant difference in VFA production and ORP values between the temperature of 29 ℃ and 37 ℃ in this study.

5 Recommendations

1. Anaerobic digester with recycling should be considered to enable SRT to fall in the range 1 – 3 days in order to entirely suppress methane production. HRT was the same as SRT in this study since there was no recycling and this may contribute to solids over loading.

2. Primary sludge from domestic wastewater treatment plants could be used instead of the soy flour feed solution. Soy flour contains more protein as compared to the domestic wastewater. Soy flour could generate high buffer capacity arising from ammonia or carbon dioxide.

3. Temperature effects on the amount of VFA generated and ORP values could be investigated more closely. The chemical results of investigating parameters such as VFA production and ORP at temperatures of 29 ℃ and 37 ℃ were not significantly different in this study. Thus, a variety of temperatures could be pursued to identify the relationship between VFAs and ORP as a function of temperature.

4. The investigation of the relationship between other products generated and ORP in acid-phase anaerobic digesters could be useful. VFAs are the most common products in acid-phase anaerobic digester; however, if there is a relationship between VFAs and another end product (i.e. ethanol, lactic acid and gases such as CO_2, H_2S and H_2) it would be useful to know this.

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Appendices

A.1 Soy flour nutrient values and weights

Nutrient	Units	Value per 100 grams	Number of Data Points	Std. Error
Proximates				
Water	g	5.16	5	
Energy	kcal	436	0	
Energy	kj	1824	0	
Protein	g	34.54	4	
Total lipid (fat)	g	20.65	2	
Ash	g	4.46	1	
Carbohydrate, by difference	g	35.19	0	
Fiber, total dietary	g	9.6	0	
Sugars, total	g	7.50	0	
Minerals				
Calcium, Ca	mg	206	4	
Iron, Fe	mg	6.37	3	
Magnesium, Mg	mg	429	2	
Phosphorus, P	mg	494	1	
Potassium, K	mg	2515	2	
Sodium, Na	mg	13	2	
Zinc, Zn	mg	3.92	2	
Copper, Cu	mg	2.920	2	
Manganese, Mn	mg	2.275	2	
Selenium, Se	mcg	7.5	0	

Source: Nutrients database for standard reference of United States Department of Agriculture (http://www.nal.usda.gov/fnic/foodcomp/search/)

A.2 Data with a 10 day SRT for digester 1

	Digester 1											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic(mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
1	2/11/06	2276.9	2400.4	2382.1	2233.1	5846.0	-340	4.90				
2	3/11/06	2470.9	2633.6	2630.8	2364.0	6398.5	-340	4.90				
5	6/11/06	2259.3	2460.6	2456.1	2262.6	5927.6	-340	4.94		21888	14870	29.4
7	8/11/06	2309.7	2599.9	2688.6	2406.4	6249.4	-318	4.94	1100	21264	11600	26.5
9	10/11/06	2345.5	2680.4	2744.1	2474.0	6388.2	-320	4.94	1120	20788	15500	26.6
12	13/11/06	2397.3	2571.2	2656.0	2588.5	6291.5	-322		1120	21212	11700	
14	15/11/06	2114.8	2322.7	2490.2	2303.4	5694.5	-320	4.84		21628	14100	28.4
16	17/11/06	2665.8	1810.3	1786.5	986.3	5350.7	-320	4.95	1170	22508	15000	28.5
19	20/11/06	2543.0	1649.2	1554.5	815.3	4939.2	-312	4.95	1170	21756	14850	26.7
21	22/11/06	2419.9	1522.2	1526.5	816.7	4694.0	-314	4.94	1020	16832	13300	31.2
23	24/11/06	2339.9	1480.3	1567.3	656.7	4607.9	-309	4.97	1160	17632	14800	31.6
26	27/11/06	2489.4	1680.0	1950.2	1001.0	5180.2	-312	4.96	1280	16908	12600	29.4
28	29/11/06	2958.2	1981.3	2171.8	1139.7	6044.2	-312	4.94	1160	17672	12800	29.6
30	1/12/06	2472.9	2997.9	2220.0	2101.8	6415.8	-318	4.95	1140	18024	13400	28.9
33	4/12/06	1920.6	2357.9	1666.6	1958.2	4967.6	-315	4.94	1140		14050	27.7
34	5/12/06	2293.1	3157.1	3055.3	2193.3	6934.3	-314	4.95	1140	17952	14000	28.2

A.3 Data with a 10 day SRT for digester 2

	Digester 2											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
1	2/11/06	2375.3	2554.7	2578.2	2376.5	6203.1	-395	4.90				
2	3/11/06	2459.5	2652.3	2624.8	2393.2	6398.2	-395	4.90				
5	6/11/06	2406.0	2683.3	2692.7	2419.4	6416.1	-390	4.99		24852	14810	30.8
7	8/11/06	2417.9	2709.1	2782.7	2499.2	6510.2	-389	4.97	1150	22204	13500	28.5
9	10/11/06	2253.6	2603.0	2664.1	2464.7	6179.1	-388	4.97	1190	21420	14800	28.6
12	13/11/06	2260.9	2566.1	2641.2	2516.4	6140.9	-388		1190	21836	14500	
14	15/11/06	2198.0	2499.6	2577.9	2413.1	5980.9	-390	5.00		19141	12350	30.7
16	17/11/06	2377.4	1534.0	1467.2	807.3	4621.3	-394	5.00	1280	19404	14750	30.9
19	20/11/06	2474.8	1549.5	1461.5	795.4	4727.4	-394	4.98	1210	19884	14850	28.8
21	22/11/06	2422.9	1567.6	1549.6	888.8	4750.1	-388	4.94	1130	19668	14300	30.7
23	24/11/06	2666.3	1549.6	1448.4	779.9	4909.9	-389	4.99	1150	19436	14300	31.0
26	27/11/06	2490.1	1476.6	1459.9	937.9	4682.4	-384	4.99	1340	19508	14400	28.8
28	29/11/06	3004.8	1721.4	1613.2	1164.2	5500.1	-378	4.99	1260	19656	14200	29.4
30	1/12/06	2439.4	2943.4	2293.6	2224.4	6388.4	-382		1280	19620	13500	
33	4/12/06	2011.0	2430.2	1779.2	2026.9	5193.4	-392	4.97	1160		14400	27.6
34	5/12/06	2173.9	2941.1	2862.7	2117.3	6508.8	-396	4.97	1180	19724	12500	27.9

A.4 Data with a 5 day SRT for digester 1

Digester 1											
Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
14/12/06	1827.0	1578.8	1259.3	643.7	4343.4	-332	4.93		20652	13000	27.3
15/12/06	2043.8	2072.0	1704.4	770.9	5338.1	-322	4.90	1140	20420	11750	27.8
18/12/06	2169.9	1973.0	1727.0	827.0	5432.4	-302	4.89	1000	21180	13100	26.1
20/12/06	1934.5	1699.4	1391.5	734.2	4692.0	-298	4.87	920	21948	12100	23.4
22/12/06	1861.2	1586.1	1316.3	652.8	4427.7	-275	4.88	800	22456	11350	17.2
27/12/06	1972.2	1515.3	1161.3	604.2	4347.2	-255	4.78	560	22944	11000	23.9
29/12/06	1508.7	1600.4	1321.1	695.2	4115.1	-255	4.80	600	24648	12400	21.3
1/01/07	1628.9	1638.5	1384.0	503.3	4196.1	-250	4.77	520	23980	12950	18.3
3/01/07	1275.0	1352.6	1092.1	400.7	3351.2	-240	4.74	460	23660	11250	19.1
5/01/07	1724.6	1592.1	1249.9	588.3	4212.8	-268	4.73	440	24004	11400	20.5
8/01/07	1240.5	1584.0	1301.1	721.3	3835.3	-280	4.75	440	24496	12200	21.0
10/01/07	1433.9	1646.6	1406.9	728.3	4155.6	-210	4.76	320	24812	12850	21.4
12/01/07	1325.3	1528.4	1330.8	713.1	3890.4	-200	4.75	500	25772	10800	21.8
15/01/07	1307.2	1341.4	1127.9	648.2	3544.3	-200	4.70	400	24228	11550	22.7
17/01/07	1365.5	1607.7	1358.9	739.0	4029.2	-228	4.76	600	24112	11900	21.9
19/01/07	1181.8	1461.2	1263.8	718.2	3649.8	-235	4.74	460	24456	12200	24.5
22/01/07	1753.0	1657.7	1614.5	643.8	4575.5	-268	4.75	540	24068	11950	25.7
26/01/07	1490.5	1517.6	1334.2	714.6	4050.0	-255	4.76	620	24764	12400	25.3
29/01/07	1803.3	1560.5	1403.6	700.9	4436.8	-285	4.78	660	22196	12250	25.7

A.5 Data with a 5 day SRT for digester 2

Digester 2											
Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
14/12/06	1815.9	1458.9	1373.6	691.5	4341.1	-390	4.94		20156	11250	27.5
15/12/06	1850.1	1795.1	1462.4	716.5	4723.1	-388	4.93	1140	19884	11900	28.2
18/12/06	1942.1	1946.0	1833.2	911.0	5304.4	-364	4.91	1060	20176	11900	26.8
20/12/06	2005.6	1977.7	1680.7	827.7	5240.7	-378	4.91	880	20668	13100	25.5
22/12/06	1886.0	1738.5	1540.6	717.9	4767.2	-385	4.90	860	21456	11650	19.4
27/12/06	2094.1	1656.3	1380.0	716.0	4798.2	-350	4.82	660	21400	12650	25.7
29/12/06	1306.2	1371.8	1135.3	468.9	3467.6	-372	4.83	680	21828	13550	23.6
1/01/07	1658.3	1564.4	1269.7	624.3	4158.7	-335	4.79	580	22004	12650	20.3
3/01/07	1418.4	1315.7	1007.3	468.3	3446.7	-338	4.76	500	22060	11350	20.8
5/01/07	1553.3	1596.3	1241.4	438.7	3951.1	-352	4.75	500	22760	12750	22.0
8/01/07	1250.9	1472.6	1154.3	751.8	3673.3	-375	4.78	500	23160	13000	23.0
10/01/07	1566.7	1471.2	1193.5	732.8	4003.5	-355	4.79	480	23692	11650	23.6
12/01/07	1334.4	1379.8	1126.2	701.5	3632.7	-350	4.80	640	22620	10800	24.5
15/01/07	1238.7	1389.8	1162.8	741.8	3593.9	-325	4.78	580	22816	11100	25.2
17/01/07	1328.3	1254.4	1075.3	687.6	3482.2	-355	4.83	680	22516	11300	24.0
19/01/07	1083.2	1298.0	1095.7	727.1	3309.5	-368	4.78	660	22564	11300	24.8
22/01/07	1484.8	1311.7	1242.7	607.1	3751.8	-350	4.85	760	22720	11950	27.2
26/01/07	1225.5	1245.3	1131.1	708.9	3422.5	-374	4.87	760	22672	12000	26.1
29/01/07	1488.8	1282.5	1198.1	694.1	3753.1	-384	4.88	840	20252	11700	26.4

A.6 Data with a 15 day SRT for digester 1

	Digester 1											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
103	16/02/07	1701.1	1399.8	1485.6	696.9	3848.1	-287	4.90	960	23288	12800	25.6
106	19/02/07						-260	4.83	1180	22968	13150	26.3
108	21/02/07	2274.9	1681.1	1616.8	717.6	4739.4	-240	4.82	900	22940	13500	27.1
110	23/02/07	2126.0	1604.3	1619.9	730.8	4530.3	-245	4.90	1160	22880	13650	27.3
112	26/02/07	2024.5	1506.1	1594.7	694.9	4332.1	-273	4.88	1020	23224	13100	26.1
114	28/02/07	1830.8	1451.9	1576.6	709.3	4082.1	-285	4.92	1160	23220	13600	24.6
116	2/03/07	2243.8	1636.7	1724.1	773.0	4745.5	-258	4.88	1020	22840	13700	25.7
119	5/03/07	1814.5	1444.3	1527.5	690.5	4026.2	-260	4.87	940	22092	13650	26.3
121	7/03/07	2025.6	1532.8	1614.5	735.3	4368.3	-295	4.88	980	21036	13450	27.2
123	9/03/07	2298.1	1644.2	1743.7	768.5	4819.2	-280	4.89	1000		14050	26.0
126	12/03/07	1908.0	1506.1	1617.7	768.3	4231.3	-240	4.92	960	21340	12650	27.2
128	14/03/07	2120.4	1543.1	1702.5	788.8	4531.4	-282	4.90	920	22528	12250	23.3
130	16/03/07	1865.8	1447.7	1603.8	728.0	4132.3	-297	4.87		20532	12850	26.4
133	19/03/07	1913.0	1438.9	1625.7	766.1	4187.3	-290	4.86	800	22208	13150	23.0
135	21/03/07	1948.1	1367.7	1446.8	658.3	4042.7	-295	4.84	900	21216	11900	25.5
137	23/03/07	2033.1	1470.4	1547.9	693.6	4279.8	-305	4.91	960	21404	13900	29.4
140	26/03/07	2374.6	1621.8	1667.7	726.9	4825.8	-307	4.92	1060	22212	14450	29.8

A.7 Data with a 15 day SRT for digester 2

	Digester 2											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
103	16/02/07	1753.3	1366.6	1441.6	676.3	3843	-300	4.89	920	24372	12750	26.0
106	19/02/07						-280	4.84	1060	23988	13300	26.7
108	21/02/07	2030.4	1516.8	1567.4	757.8	4328	-307	4.84	920	24132	12950	27.9
110	23/02/07	1990.8	1343.3	1307.5	582.9	3971	-300	4.91	1020	23112	13150	27.5
112	26/02/07	2149.1	1532.3	1666.4	743.9	4527	-276	4.90	1080	23132	14250	27.3
114	28/02/07	1711.6	1227.4	1355.6	620.7	3630	-288	4.93	1080	23116	13300	25.7
116	2/03/07	2140.9	1454.6	1581.4	733.6	4398	-287	4.90	1020	22224	13750	26.2
119	5/03/07	1786.1	1329.0	1459.4	662.6	3858	-287	4.91	1040	21972	13700	26.7
121	7/03/07	1798.3	1294.8	1380.2	620.6	3788	-287	4.93	1120	21512	12850	28.2
123	9/03/07	2214.6	1578.2	1676.6	755.7	4636	-283	4.94	1060		14000	26.7
126	12/03/07	1830.2	1430.7	1613.9	750.9	4090	-305	4.98	1080	21656	12650	27.5
128	14/03/07	2026.8	1535.4	1603.0	763.3	4364	-245	4.93	1020	22872	13050	23.7
130	16/03/07	1938.5	1559.9	1604.2	783.2	4296	-308	4.92	1000	21860	13100	27.2
133	19/03/07	2032.7	1711.9	1733.5	849.4	4602	-290	4.92	980	22180	12800	24.3
135	21/03/07	2014.1	1620.1	1607.5	802.6	4423	-297	4.93	1000	21452	13500	27.3
137	23/03/07	1991.0	1550.3	1516.3	743.7	4281	-317	4.90	1000	21224	13300	28.2
140	26/03/07	2217.5	1682.0	1646.5	811.6	4703	-305	4.93	1060	21804	14000	28.8

A.8 Data with a 12 day SRT for digester 1

	Digester 1											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
156	11/04/07	1887.0	1509.1	1484.8	753.8	4122.1	-325	4.93	1040	20896	13900	25.5
158	13/04/07	1980.6	1542.8	1493.4	776.8	4248.9	-325	4.93	1020	20936	12500	24.9
161	16/04/07	1983.4	1525.6	1467.3	780.0	4219.9	-320	4.89	960	21164	13850	25.4
163	18/04/07	2083.4	1765.2	1777.1	977.5	4725.3	-325	4.90	1020	20828	14650	25.6
165	20/04/07	1958.4	1535.7	1447.8	796.1	4189.9	-320	4.88	960	21220	14000	25.9
168	23/04/07	2305.8	1715.2	1567.2	878.7	4764.1	-317	4.87	920	21564	13300	25.4
170	25/04/07	2185.3	1645.2	1592.5	884.3	4604.2	-314	4.88	880	21728	13800	26.0
172	27/04/07	2253.3	1692.4	1616.0	834.8	4726.5	-316	4.88	920	21644	14450	26.3
175	30/04/07	2365.2	1657.7	1552.0	894.9	4766.6	-316	4.86	920	21708	12500	25.5
177	2/05/07	2186.3	1586.6	1511.2	847.5	4502.2	-312	4.85	900	21460	13250	26.0
179	4/05/07	2208.5	1523.3	1396.4	765.0	4394.9	-312	4.79	700	21624	12450	24.6
182	7/05/07	2252.9	1535.6	1447.6	814.1	4484.1	-314	4.81	680	21524	13600	26.2
184	9/05/07	2258.8	1585.8	1527.8	889.4	4585.4	-315	4.81	720	21500	12800	25.7

A.9 Data with a 12 day SRT for digester 2

	Digester 2											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
156	11/04/07	1747.7	1428.9	1422.2	741.4	3875	-374	4.94	1060	20856	12950	27.4
158	13/04/07	2140.6	1814.4	1977.3	1139.1	4959	-380	4.94	1000	20968	12750	26.5
161	16/04/07	1925.1	1470.0	1430.0	779.6	4091	-394	4.92	1020	21016	14000	26.8
163	18/04/07	1860.7	1483.9	1488.3	833.3	4078	-395	4.93	1000	20728	14150	27.0
165	20/04/07	1792.5	1423.6	1354.3	765.4	3869	-395	4.91	1000	21300	14200	26.7
168	23/04/07	2193.2	1651.1	1558.7	916.0	4594	-398	4.92	1000	21216	13500	26.4
170	25/04/07	2197.0	1709.1	1644.7	1000.9	4703	-390	4.95	1060	20688	14750	26.9
172	27/04/07	2044.5	1614.1	1545.0	942.3	4406	-397	4.97	1180	20552	14650	27.5
175	30/04/07	2295.2	1626.8	1468.3	872.3	4615	-393	4.91	1020	21128	13700	26.5
177	2/05/07	2111.3	1555.6	1471.8	892.7	4375	-390	4.95	1040	21124	13350	27.4
179	4/05/07	2092.1	1487.2	1330.2	748.6	4204	-398	4.88	840	21352	11300	26.3
182	7/05/07	2174.6	1618.1	1490.7	913.8	4502	-398	4.86	860	21176	14250	27.2
184	9/05/07	2024.8	1513.1	1355.4	836.5	4175	-400	4.86	860	21168	12200	26.9

A.10 Data with an 8 day SRT for digester 1

	Digester 1											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
193	18/05/07	1829.0	1371.4	1325.7	779.4	3844.2	-337	4.84	560	20692	13250	26.2
196	21/05/07	1852.3	1285.2	1279.3	744.3	3765.8	-330	4.88	720	19832	13200	27.1
198	23/05/07	1849.5	1266.5	1304.0	749.7	3764.7	-320	4.91	730	20716	13150	26.7
200	25/05/07	1849.9	1300.1	1376.9	782.7	3842.1	-320	4.91	760	21196	12750	25.7
203	28/05/07	1979.9	1324.1	1320.4	729.3	3953.0	-326	4.88	680	20932	12700	25.7
205	30/05/07	2006.5	1310.4	1302.8	715.0	3956.5	-317	4.87	760	21368	13700	25.7
207	1/06/07	1898.3	1143.9	1057.8	541.3	3546.4	-322	4.85	620	21192	12550	26.3
210	4/06/07	1796.7	1273.9	1306.9	724.5	3719.9	-320	4.85	620	21308	12800	25.1
212	6/06/07	2069.5	1606.8	1687.5	955.6	4521.9	-319	4.83	560	20692	13200	25.4
214	8/06/07	1839.6	1295.3	1336.7	745.9	3800.5	-318	4.82	580	21360	13200	25.4
217	11/06/07	1741.1	1315.6	1344.1	715.7	3723.4	-316	4.84	820	20688	12600	24.8

A.11 Data with an 8 day SRT for digester 2

	Digester 2											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
193	18/05/07	1972.0	1409.3	1302.0	764.1	4002	-417	4.84	680	23184	12800	27.1
196	21/05/07	2001.5	1387.1	1279.6	746.7	3998	-418	4.88	880	23272	13850	27.7
198	23/05/07	1980.5	1448.0	1387.7	810.1	4100	-409	4.94	860	23376	13950	27.4
200	25/05/07	1999.2	1407.3	1350.1	770.3	4060	-416	4.93	870	23304	12850	27.1
203	28/05/07	2082.7	1463.3	1429.9	795.9	4243	-395	4.91	820		13200	26.8
205	30/05/07	1540.2	1077.6	1118.9	523.9	3176	-412	4.91	800	17396	11500	27.1
207	1/06/07	1981.5	1257.5	1205.0	614.2	3822	-407	4.92	820	18280	11750	27.5
210	4/06/07	1735.9	1228.6	1315.0	718.5	3628	-388	4.92	780	21296	12050	26.9
212	6/06/07	2104.7	1530.9	1710.4	966.8	4511	-391	4.90	840	20040	12950	27.3
214	8/06/07	1667.8	1150.2	1260.0	672.2	3459	-367	4.91	760	22172	13400	27.1
217	11/06/07	1677.8	1192.7	1285.9	677.3	3521	-381	4.92	1000	21064	12800	27.2

A.12 Average values of data measured with different SRTs in digester 1 and 2

Digester1							
HRT/SRT (day)	ORP (mv)	Total VFA as Acetic (mg/l)	VSS (mg/l)	SCOD (mg/l)	Alkalinity (mg/l)	pH	Tem (℃)
5	-245	3645	24153	11936	509	4.8	22.4
8	-319	3878	21101	13008	660	4.8	25.5
10	-315	5556	17503	13691	1150	4.9	29.0
12	-316	4606	21661	13269	830	4.8	25.7
15	-283	4385	21975	13261	972	4.9	25.5

Digester2							
HRT/SRT (day)	ORP (mv)	Total VFA as Acetic (mg/l)	VSS (mg/l)	SCOD (mg/l)	Alkalinity (mg/l)	pH	Tem (℃)
5	-356	3365	22362	11982	630	4.9	24.5
8	-391	3686	20041	12408	833	4.9	27.2
10	-389	5400	19782	14005	1219	5.0	29.5
12	-396	4579	20896	14150	983	4.9	26.9
15	-287	4241	22174	13244	1044	4.9	26.2

A.13 Data with 37 ℃ for digester 1

	Digester 1											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
51	8/08/2007	2468.1	1163.3	1045.0	419.5	4123.2	-355	4.78	890	22032	13850	37.5
53	10/08/2007	2452.6	1340.0	1349.8	583.4	4458.7	-355	4.77	840	22616	13600	38.0
56	13/08/2007	2928.9	1688.7	1740.5	824.3	5483.8	-357	4.79	930	21992	13450	39.0
58	15/08/2007	2818.8	1864.4	2091.3	1115.2	5755.2	-356	4.81	970	21596	13900	35.0
60	17/08/2007	3023.4	1510.5	1440.9	859.2	5229.7	-353	4.88	1050	21656	14600	37.0
63	20/08/2007	2675.9	1429.9	1387.2	731.0	4780.3	-356	4.89	1250	21324	14800	37.3
65	22/08/2007	2803.4	1533.1	1426.9	820.4	5018.5	-355	4.91	1200	21592	16550	37.0
67	24/08/2007	2662.5	1461.6	1355.4	775.7	4770.9	-359	4.90	1160	21384	14950	37.0
70	27/08/2007	2514.3	1273.4	1134.4	629.9	4319.6	-364	4.89	1400	21752	14250	37.0
72	29/08/2007	2797.7	1447.5	1332.2	824.1	4878.9	-365	4.96	1560	21116	15350	37.5
74	31/08/2007	2769.9	1394.7	1284.9	801.1	4776.1	-362	4.93	1400	21112	14700	38.0

A.14 Data with 37 ℃ for digester 2

	Digester 2											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
51	8/08/2007	2547.8	1294.4	1236.2	535.5	4439.5	-356	4.78	950	22204	13650	37.5
53	10/08/2007	2938.4	1732.9	1841.5	907.1	5598.0	-357	4.78	920	22060	14150	37.0
56	13/08/2007	3086.8	1876.4	1909.2	904.0	5908.8	-358	4.79	980	22260	12850	38.5
58	15/08/2007	2785.0	1942.7	2174.0	1136.3	5841.2	-360	4.80	1030	21564	13950	36.0
60	17/08/2007	2835.2	1652.5	1793.7	1002.9	5397.1	-357	4.89	1080	21688	13400	36.0
63	20/08/2007	2719.1	1609.3	1627.4	825.2	5132.6	-365	4.88	1140	21460	14450	37.0
65	22/08/2007	2531.8	1523.6	1445.2	744.7	4751.7	-365	4.86	1130	21188	15100	36.0
67	24/08/2007	2528.0	1583.3	1547.2	821.7	4865.9	-364	4.86	1100	21436	14200	37.0
70	27/08/2007	2486.4	1449.3	1373.6	724.2	4597.3	-369	4.85	1240	21660	13450	36.5
72	29/08/2007	2751.5	1646.5	1595.8	934.9	5173.6	-369	4.92	1440	20728	15250	37.0
74	31/08/2007	2779.0	1523.1	1400.1	774.7	4967.7	-364	4.88	1180	20672	14600	37.5

A.15 Data with 14 ℃ for digester 1

	Digester 1											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
91	10/09/2007	2571.9	1038.4	977.8	511.6	4080.0	-340	4.91	1000	18960	13550	19.0
93	12/09/2007	2496.7	900.2	785.0	337.8	3761.3	-260	4.81	700	21716	12700	16.0
95	14/09/2007	2526.1	1114.6	877.4	430.2	4027.5	-265	4.69	560	21708	9150	14.3
98	17/09/2007	1763.9	585.4	466.9	145.6	2556.6	-265	4.62	120	21180	12350	14.4
100	19/09/2007	2391.5	1032.8	735.8	319.4	3730.2	-255	4.39	0	22000	12400	14.9
102	21/09/2007	1798.8	728.1	516.0	277.5	2740.6	-240	4.29	0	22596	12800	15.2
105	24/09/2007	1949.5	595.1	643.2	277.5	2870.2	-220	4.20	0	22816	11500	14.9
107	26/09/2007	1605.4	409.2	549.3	277.5	2311.4	-220	4.14	0	22252	12150	14.2
109	28/09/2007	1670.5	300.4	516.0	277.5	2265.7	-203	4.10	0	22488	12950	14.3
112	1/10/2007	1619.4	431.0	562.7	277.5	2352.1	-190	4.04	0	22808	11200	15.1
114	3/10/2007	1616.3	425.0	516.0	277.5	2312.4	-180	4.06	0	20484	13000	13.8

A.16 Data with 14 ℃ for digester 2

	Digester 2											
	Date	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
91	10/09/2007	2651.1	1237.8	1084.7	605.1	4393.6	-420	4.89	920	21980	13200	18.5
93	12/09/2007	2558.3	1170.8	996.6	531.7	4186.5	-260	4.80	540	21360	11350	15.1
95	14/09/2007	2449.0	1080.5	866.4	439.2	3915.3	-275	4.70	360	22760	8050	14.1
98	17/09/2007	1945.7	856.7	652.9	291.6	3085.1	-295	4.60	60	22252	11200	13.9
100	19/09/2007	2241.5	980.5	722.2	340.3	3528.4	-290	4.39	0	23632	11600	13.6
102	21/09/2007	1658.6	698.8	516.0	277.5	2576.7	-285	4.27	0	21704	11300	14.5
105	24/09/2007	1622.9	468.8	596.2	277.5	2409.1	-275	4.18	0	21664	10100	14.5
107	26/09/2007	1317.2	334.4	516.0	277.5	1939.9	-272	4.12	0	21616	12150	13.9
109	28/09/2007	1545.0	392.4	516.0	277.5	2214.8	-260	4.08	0	20428	11800	14.0
112	1/10/2007	1269.7	308.5	516.0	277.5	1871.4	-260	4.04	0	18976	9050	14.9
114	3/10/2007	1354.9	342.0	516.0	277.5	1983.7	-256	4.05	0	19844	12500	13.9

A.17 Average values of data measured as a function of temperature in digester 1 and 2

Digester 1											
Temperature (℃)	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
14	1710	481	551	277	2475	-209	4.14	0	22241	12267	14.6
29	2420	2139	2059	1506	5556	-315	4.94	1150	19212	13691	29.0
37	2750	1436	1337	777	4825	-359	4.91	1289	21419	15029	37.3

Digester 2											
Temperature (℃)	Acetic (mg/l)	Propionic (mg/l)	n-Butyric (mg/l)	n-Valeric (mg/l)	Total as Acetic (mg/l)	ORP (mv)	pH	Alkalinity (mg/l)	VSS (mg/l)	SCOD (mg/l)	Tem (℃)
14	1461	424	529	277	2166	-268	4.12	0	20705	11150	14.3
29	2411	2071	1923	1516	5400	-389	4.98	1218	19788	14005	29.5
37	2662	1570	1540	833	4984	-365	4.88	1187	21262	14350	36.7